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APPLICATION FOR LETTERS PATENT

for

***STREPTOCOCCUS SUI* VACCINES AND DIAGNOSTIC TESTS**

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STREPTOCOCCUS SUIIS VACCINES AND DIAGNOSTIC TESTS

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] ~~Cross-reference to Related Applications.~~ This application claims priority to, and is a continuation of, International Application No. PCT/NL99/00460, filed on July 19, 1999, designating the United States of America, the contents of which are incorporated herein by this reference, the PCT International Patent Application itself claiming priority from European Patent Office Application Serial No. 98202465.5 filed July 22, 1998 and European Patent Office Application Serial No. 98202467.1 filed July 22, 1998.

TECHNICAL FIELD

[0002] ~~Technical Field.~~ The invention relates to *Streptococcus* infections in pigs, vaccines directed against those infections, tests for diagnosing *Streptococcus* infections and bacterial vaccines. More particularly, the invention relates to vaccines directed against *Streptococcus* infections.

~~Background of the Invention~~ BACKGROUND OF THE INVENTION

[0003] ~~Streptococcus~~ *Streptococcus* species, of which a large variety cause infections in domestic animals and man, are often grouped according to Lancefield's groups. Typing according to Lancefield occurs on the basis of serological determinants or antigens that are, among others, present in the capsule of the bacterium, and allows for only an approximate determination. Often, bacteria from different groups show cross-reactivity with each other, while other *Streptococci* ~~can not~~ cannot be assigned a group-determinant at all. Within groups, further differentiation is often possible on the basis of serotyping. These serotypes further contribute to the large antigenic variability of *Streptococci*, a fact that creates an array of difficulties within diagnosis of and vaccination against *Streptococcal* infections.

[0004] Lancefield group A *Streptococcus species* ~~(GAS, (Group A Streptococci "GAS,"~~ *Streptococcus pyogenes)*, ~~pyogenes)~~ are common in children, causing nasopharyngeal infections and complications thereof. Among animals, cattle are especially susceptible to GAS infection which can cause mastitis.

[0005] Group A streptococci are the etiologic agents of streptococcal pharyngitis and impetigo, two of the most common bacterial infections in children, as well as a variety of less common, but potentially life-threatening, infections including soft tissue infections, bacteremia, and pneumonia. In addition, GAS are uniquely associated with the post-infectious autoimmune syndromes of acute rheumatic fever and post streptococcal glomerulonephritis.

[0006] Several recent reports suggest that the incidence of both serious infections due to GAS and acute rheumatic fever has increased during the past decade, focusing renewed interest on defining the attributes or virulence factors of the organism that may play a role in the pathogenesis of these diseases.

[0007] GAS produce several surface components and extracellular products that may be important in virulence. The major surface protein, M protein, has been studied in the most detail and has been ~~shown~~ convincingly shown to play a role in both virulence and immunity. Isolates rich in M protein are able to grow in human blood, a property thought to reflect the capacity of N protein to interfere with phagocytosis, and these isolates tend to be virulent in experimental animals.

[0008] Lancefield group B *Streptococcus* (~~GBS~~) (“GBS”) are most often seen ~~with~~ in cattle, causing ~~mastitis~~, mastitis; however, human infants are susceptible as well, often with fatal consequences. Group B streptococci (GBS) constitute a major cause of bacterial sepsis and meningitis among human neonates born in the United States and Western Europe and are emerging as significant neonatal pathogens in developing countries as well.

[0009] It is estimated that GBS strains are responsible for 10,000 to 15,000 cases of invasive infection in neonates in the United States alone. Despite advances in early diagnosis and treatment, neonatal sepsis due to GBS continues to carry a mortality rate of 15 to 20%. In addition, survivors of GBS meningitis have 30 to 50% incidence of long-term neurologic sequelae. ~~The~~ Over the past two decades, increasing recognition ~~over the past two decades~~ of GBS as an important pathogen for human infants has generated renewed interest in defining the bacterial and host factors important in virulence of GBS and in the immune response to GBS infection.

[0010] Particular attention has focused on the capsular polysaccharide as the predominant surface antigen of the organisms. In a modification of the system originally developed by Rebecca Lancefield, GBS strains are serotyped on the basis of ~~antigen~~ antigenic differences in their capsular

polysaccharides and the presence or absence of serologically defined C proteins. While GBS isolated from non-human sources often lack a serologically detectable capsular, a large majority of strains associated with neonatal infection belong to one of four major capsular serotypes, 1a, 1b, II or III. The capsular polysaccharide forms the outermost layer around the exterior of the bacterial cell, superficial to the cell wall. The capsule is distinct from the cell wall-associated group B carbohydrate. It has been suggested that the presence of sialic-~~acid~~ acid, in the capsule of bacteria that ~~cause meningitis~~ causes meningitis, is important for allowing these bacteria to breach the blood-brain barrier. Indeed, in ~~S. agalactiae~~ S. agalactiae, sialic acid has been shown to be critical for the virulence function of the type III capsule. The capsule of ~~S. suis~~ S. suis serotype is composed of glucose, galactose, N-acetylglucosamine, rhamnose and sialic acid.

[0011] The group B polysaccharide, in contrast to the type-specific capsule, is present on all GBS strains and is the basis for serogrouping-~~of the~~ the organisms into Lancefield's group B. Early studies by Lancefield and co-workers showed that antibodies raised in rabbits against whole GBS organisms protected mice against challenge with strains of homologous capsular type, demonstrating the central role of the capsular polysaccharide as a protective antigen. Studies in the 1970s by Baker and Kasper demonstrated that cord blood of human infants with type III GBS sepsis uniformly had low or undetectable levels of antibodies directed against the type III capsule, suggesting that a deficiency of anticapsular antibody was a key factor in susceptibility of human neonates to GBS disease.

[0012] Lancefield group C infections, such as those with *S. equi*, *S. zooepidemicus*, *S. dysgalactiae*, and ~~others~~ others, are mainly seen ~~with horse,~~ in horses, cattle and pigs, but can also cross the species barrier to humans. Lancefield group D (*S. bovis*) infections are found ~~with~~ in all mammals and some birds, sometimes resulting in endocarditis or ~~septicaemia~~ septicemia.

[0013] Lancefield groups E, G, L, P, U and V (*S. porcinus*, ~~s. canis,~~ s. canis, *s. dysgalactiae*) are found ~~with~~ in various hosts, causing neonatal infections, nasopharyngeal infections or mastitis.

~~Within Lancefield groups R, S, and T, (and with ungrouped types) S. suis is found, an important cause of meningitis, septicemia, arthritis and sudden death in young pigs. Incidentally, it can also cause meningitis in man.~~

[0014] Within Lancefield groups R, S and T (and with ungrouped types), *Streptococcus suis* is an important cause of meningitis, septicemia, arthritis and sudden death in young pigs (4, 46). Incidentally, it can also cause meningitis in man (1). *S. suis* strains are usually identified and classified by their morphological, biochemical and serological characteristics (58, 59, 46). Serological classification is based on the presence of specific antigenic polysaccharides. So far, 35 different serotypes have been described (9, 56, 14). In several European countries, *S. suis* serotype 2 is the most prevalent type isolated from diseased pigs, followed by serotypes 9 and 1. Serological typing of *S. suis* ~~is carried out~~ performed using different types of agglutination tests. In these tests, isolated and biochemically ~~characterised~~ characterized *S. suis* cells are agglutinated with a panel of 35 specific sera. These methods are very laborious and time-consuming.

[0015] Little is known about the pathogenesis of the disease caused by *S. suis*, let alone about its various serotypes such as type 2. Various bacterial components, such as extracellular and cell-membrane associated proteins, fimbriae, ~~haemagglutinins,~~ hemagglutinins, and ~~haemolysin~~ hemolysis, have been suggested as virulence factors (9, 10, 11, 15, 16, 47, 49). However, the precise role of these protein components in the pathogenesis of the disease remains unclear (37). It is well known that the ~~polysaccharidic~~ polysaccharide capsule of various Streptococci and other gram-positive bacteria plays an important role in pathogenesis (3, 6, 35, 51, 52). The capsule enables these ~~micro-organisms~~ microorganisms to resist phagocytosis and is therefore regarded as an important virulence factor. Recently, a role of the capsule of *S. suis* in the pathogenesis was suggested as well (5). However, the structure, ~~organisation~~ organization and ~~functioning~~ function of the genes responsible for capsule polysaccharide synthesis (~~eps~~) (cps) in *S. suis* is unknown. Within ~~*S. suis* serotypes~~ *suis*, serotype 1 and 2 strains can differ in virulence for pigs (41, 45, 49). Some type 1 and 2 strains are virulent, other strains are not. Because both virulent and non-virulent strains of serotype 1 and 2 strains are fully encapsulated, it may even be that the capsule is not a relevant factor required for virulence.

[0016] Attempts to control *S. suis* infections or disease are still hampered by the lack of knowledge about the epidemiology of the disease and the lack of effective vaccines and sensitive diagnostics. It is well known and generally accepted that the ~~polysaccharidic~~ polysaccharide capsule of various Streptococci and other gram-positive bacteria plays an important role in pathogenesis.

The capsule enables these ~~micro-organisms~~ microorganisms to resist phagocytosis and is therefore regarded as an important virulence factor.

[0017] Compared to encapsulated *S. suis* strains, non-encapsulated *S. suis* strains are phagocytosed by murine polymorphonuclear leucocytes to a greater degree. Moreover, an increase in thickness of capsule was noted for *in vivo* grown virulent strains while no increase was observed for avirulent strains. ~~Therefor,~~ Therefore, these data again demonstrate the role of the capsule in the pathogenesis for *S. suis* as well.

[0018] Ungrouped *Streptococcus species*, such as *S. mutans*, causing ~~carries with~~ caries in humans, ~~S. S. uberis~~, causing mastitis ~~with in~~ in cattle, and *S. pneumonia*, causing major infections in humans, and *Enterococcus faecilalis* and *E. faecium*, further ~~contributed~~ contribute to the large group of Streptococci.

[0019] *Streptococcus pneumoniae* (the pneumococcus) is a human pathogen causing invasive diseases, such as pneumonia, ~~bacteraemia,~~ bacteremia, and meningitis. Despite the availability of antibiotics, pneumococcal infections remain common and can still be fatal, especially in high-risk groups, such as young children and elderly people. Particularly in developing countries, many children under the age of five years die each year from pneumococcal pneumonia. ~~S.~~ *S. pneumoniae* is also the leading cause of otitis media and sinusitis. These infections are less serious, but nevertheless incur substantial medical costs, especially when leading to complications, such as permanent deafness. The normal ecological niche of the pneumococcus is the nasopharynx of man. The entire human population is ~~colonised~~ colonized by the pneumococcus at one time or another, and at a given time, up to 60% of individuals may be carriers. Nasopharyngeal carriage of pneumococci by man is often accompanied by the development of protection ~~to~~ against infection by the same serotype. Most infections do not occur after prolonged carriage but follow ~~the acquisition of exposure to~~ recently acquired strains. Many bacteria contain surface polysaccharides ~~which that~~ that act as a protective layer against the environment. Surface polysaccharides of pathogenic bacteria usually make the bacteria resistant to the defense mechanisms of the host, ~~e.g., for example,~~ the lytic action of serum or phagocytosis. In this respect, the serotype-specific capsular polysaccharide ~~(CP)~~ (“CP”) of *Streptococcus pneumoniae*, is an important virulence factor. Unencapsulated strains are avirulent, and antibodies directed against the CP are protective. Protection is serotype specific; each

serotype has its own, specific CP structure. Ninety different capsular serotypes have been identified. Currently, CPs of 23 serotypes are included in a vaccine.

[0020] Vaccines directed against *Streptococcus* infections ~~in general~~ typically aim at ~~utilising~~ to utilize an immune response directed against the polysaccharide capsule of the various *Streptococcus species*, especially since the capsule is considered a ~~main~~ primary virulence factor for these bacteria. ~~The capsule, during~~ During infection, the capsule provides resistance ~~to~~ against phagocytosis and thus ~~promotes the escape of~~ protects the bacteria from the immune system of the host, ~~protecting the bacteria by~~ host, and from elimination by macrophages and neutrophils.

[0021] The capsule particularly confers the bacterium resistance to complement-mediated opsonophagocytosis. In addition, some bacteria express capsular polysaccharides (CPs) that mimic host molecules, thereby avoiding the immune system of the host. Also, even when the bacteria have been phagocytosed, intracellular killing is hampered by the presence of a capsule.

[0022] It is ~~in general~~ generally thought that ~~only when the host has antibodies or other serum factors directed against capsule antigens,~~ the bacterium will get ~~recognised~~ recognized by the immune system through the anticapsular-antibodies or serum-factors bound to its ~~capsule,~~ capsule and will, through ~~opsonisation,~~ opsonization, get phagocytosed and killed only when the host has antibodies or other serum factors directed against capsule antigens.

[0023] However, these antibodies are serotype-specific, and will often only confer protection against only one of the many serotypes known within a group of *Streptococci*.

[0024] For example, current commercially available *S. suis* vaccines, which are ~~in general~~ generally based on whole-cell-bacterial preparations, or on capsule-enriched fractions of *S. suis*, confer only limited protection against heterologous strains. Also, the current pneumococcal ~~vaccine,~~ vaccine that was licensed in the United States in 1983, consists of purified CPs of 23 pneumococcal serotypes whereas at least 90 CP types exist.

[0025] The composition of this pneumococcal vaccine was based, on the frequency of the occurrence of disease isolates in the US and cross-reactivity between various serotypes. Although this vaccine protects healthy adults against infections caused by serotypes included in the vaccine, it fails to raise a protective immune response in infants younger than 18 months and it is less effective

in elderly people. In addition, the vaccine confers only limited protection in patients with immunodeficiencies and ~~haematology~~ hematology malignancies.

[0026] ~~In the light of above,~~ Thus, improved vaccines are needed against *Streptococcus* infections. Much attention is ~~being paid at~~ directed toward producing CP vaccines by producing the relevant polysaccharides via chemical or recombinant means. However, chemical synthesis of polysaccharides is costly, and capsular polysaccharide synthesis by recombinant means necessitates knowledge about the relevant genes, which ~~are~~ is not always ~~available~~ available, and ~~need to be~~ needs to be determined for ~~each and every~~ relevant serotype.

DISCLOSURE OF THE INVENTION

[0027] The invention provides an isolated or recombinant nucleic acid encoding a capsular (*cps*) gene cluster of *Streptococcus suis*. Biosynthesis of capsule polysaccharides ~~in general has~~ has generally been studied in a number of Gram-positive and Gram-negative bacteria (32). In Gram-negative bacteria, but also in a number of ~~gram positive~~ Gram-positive bacteria, genes which are involved in the biosynthesis of polysaccharides are clustered at a single locus.

[0028] *Streptococcus suis* capsular ~~genes~~ genes, as provided by the ~~invention~~ invention, show a common genetic ~~organisation~~ organization involving three distinct regions. The central region is serotype specific and encodes enzymes responsible for the synthesis and ~~polymerisation~~ polymerization of the polysaccharides. ~~This~~ The central region is flanked by two regions conserved in *Streptococcus suis* which encode proteins for common ~~functions~~ functions, such as transport of the polysaccharide across the cellular membrane. However, ~~in between~~ between species, only low homologies exist, hampering easy comparison and detection of seemingly similar genes. Knowing the nucleic acid encoding the flanking regions allows type-specific determination of nucleic acid of the central region of *Streptococcus suis* ~~serotypes, as for example~~ serotypes as, for example, described ~~in the experimental part of the description of the invention.~~ herein.

[0029] The invention provides an isolated or recombinant nucleic acid encoding a capsular gene cluster of *Streptococcus suis* or a gene or gene fragment derived thereof. Such a nucleic acid ~~is, for example~~ is, for example, provided by ~~hybridising~~ hybridizing chromosomal DNA derived from any one of the *Streptococcus suis* serotypes to a nucleic acid encoding a gene derived from a

Streptococcus suis serotype 1, 2 or 9 capsular gene cluster, as provided by the invention (~~see (see,~~ for example example, Tables 4 and 5) and cloning of (type-specific) genes ~~as for example as, for example,~~ described in the experimental part of the description herein. At least 14 open reading frames are identified. Most of the genes belong to a single transcriptional unit, identifying a ~~co-ordinate~~ coordinate control of these genes, ~~they, genes.~~ The genes, and the enzymes and proteins they encode, act in concert to provide the capsule with the relevant polysaccharides.

[0030] The invention provides *cps* genes and proteins encoded thereof involved in regulation (CpsA), chain length determination (CpsB, C), export (CpsC) and biosynthesis (CpsE, F, G, H, J, K). Although, at first glance, the overall ~~organisation~~ organization seemed ~~at first glance~~ to be similar to that of the *cps* and *eps* gene clusters of a number of Gram-positive bacteria (19, 32, 42), overall homologies are low (~~see (see,~~ table 3). The region involved in biosynthesis is located at the ~~centre~~ center of the gene cluster and is flanked by two regions containing genes with more common functions.

[0031] The invention provides an isolated or recombinant nucleic acid encoding a capsular gene cluster of *Streptococcus suis* serotype ~~2 or 2,~~ or a gene or gene fragment derived thereof, preferably as identified in ~~Figure FIG.~~ FIG. 3. Genes in this gene cluster are involved in polysaccharide biosynthesis of capsular components and antigens. For a further description of such genes ~~see for example see,~~ for example, Table 2 of the description, for example 2. For example, a *cpsA* gene is provided functionally encoding regulation of capsular polysaccharide synthesis, whereas *cpsB* and *cpsC* are functionally involved in ~~chain-in-chain~~ chain-in-chain length determination. Other genes, such as *cpsD*, E, F, G, H, I, J, K and related genes, are involved in polysaccharide ~~syntheses,~~ functioning for example synthesis, functioning, for example, as glucosyl- or glycosyltransferase. The *cpsF*, G, H, I, J genes encode more type-specific proteins than the flanking genes which are found more-or-less conserved throughout the species and can ~~serve as base~~ as a base for selection of primers or probes in PCR-amplification or ~~cross-hybridisation~~ cross-hybridization experiments for subsequent cloning.

[0032] ~~For example, the~~ The invention further provides an isolated or recombinant nucleic acid encoding a capsular gene cluster of *Streptococcus suis* serotype 1 or a gene or gene fragment derived thereof, preferably as identified in ~~Figure FIG.~~ FIG. 4.

[0033] In addition, the invention provides an isolated or recombinant nucleic acid encoding a capsular gene cluster of *Streptococcus suis* serotype 9 or a gene or gene fragment derived thereof, preferably as identified in ~~Figure~~ FIG. 5.

[0034] Furthermore, the invention ~~provides for example~~ provides, for example, a fragment ~~of the *cps* locus, or parts thereof of the *eps* locus, involved in the capsular polysaccharide biosynthesis, biosynthesis of *S. suis*, *suis* exemplified in the experimental part for serotype herein for serotypes 1, 2 or 9, and allows easy identification or detection of related fragments derived of other serotype serotypes of *S. suis*.~~

[0035] The invention provides a nucleic acid probe or primer derived from a nucleic acid according to the invention allowing species or ~~serotype specific~~ serotype-specific detection of *Streptococcus suis*. Such a probe or primer (~~herein used interchangeably herein~~) ~~is for example is, for example,~~ a DNA, RNA or PNA (peptide nucleic acid) probe ~~hybridising~~ hybridizing with capsular nucleic acid as provided by the invention. ~~Species specific~~ Species-specific detection is provided preferably by selecting a probe or primer sequence from a species-specific region (~~e.g.~~ (e.g. flanking region) whereas ~~serotype specific~~ serotype-specific detection is provided preferably by selecting a probe or primer sequence from a type-specific region (~~e.g.~~ (e.g. central region) of a capsular gene cluster as provided by the invention. Such a probe or primer can be used in a further unmodified form, ~~for example~~ example, in ~~cross hybridisation~~ cross-hybridization or polymerase-chain reaction (PCR) experiments ~~as for example~~ as, for example described in the experimental part of the description of the invention. ~~Herein the~~ herein. The invention provides the isolation and molecular ~~characterisation~~ characterization of additional ~~type specific~~ type-specific *cps* genes of *S. suis* types 1 and 9. In addition, we describe the genetic diversity of the *cps* loci of serotypes 1, 2 and 9 among the 35 *S. suis* serotypes ~~yet known~~ known. Type-specific probes are identified. Also, a type-specific-PCR ~~for for example~~ PCR, for example, for serotype 9 is 9, is provided, being a rapid, reliable and sensitive ~~assay, which is~~ assay used directly on nasal or tonsillar swabs or other samples of infected or carrier animals.

[0036] The invention also provides a probe or primer according to the invention ~~further provided~~ with at least one reporter molecule. Examples of reporter molecules are manifold and known in the art, ~~for example~~ art; for example, a reporter molecule can ~~comprise~~ include additional

nucleic acid provided with a specific sequence (~~e.g. (e.g. oligo-dT) hybridising~~ hybridizing to a corresponding sequence ~~to which hybridisation~~ in which hybridization can easily be ~~detected for example~~ detected, ~~for example~~, because it has been ~~immobilised~~ immobilized to a solid support.

[0037] Yet other reporter molecules ~~comprise~~ include chromophores, ~~e.g. e.g.~~ fluorochromes for visual detection, ~~for example~~ example, by light microscopy or fluorescent ~~in situ hybridisation (FISH)~~ in situ hybridization (“FISH”) techniques, or ~~comprise~~ include an enzyme such as horseradish peroxidase for enzymatic detection, ~~e.g. e.g.~~ in enzyme-linked assays ~~(EIA)~~ (“EIA”). Yet other reporter molecules ~~comprise~~ include radioactive compounds for detection in ~~radiation-based assays~~ radiation-based assays.

[0038] In a preferred embodiment of the invention, at least one probe or primer according to the invention is provided ~~(labelled)~~ (labeled) with a reporter molecule and a quencher molecule, ~~providing together with an~~ unlabeled probe or primer in a PCR-based test allowing rapid detection of specific ~~hybridisation~~ hybridization.

[0039] The invention further provides a diagnostic test or test kit ~~comprising~~ including a probe or primer as provided by the invention. Such a test or test kit is, ~~for example~~ example, a ~~cross hybridisation~~ cross-hybridization test or PCR-based test, ~~is~~ test advantageously used in rapid detection and/or serotyping of *Streptococcus suis*.

[0040] The invention ~~furthermore~~ further provides a protein or fragment thereof encoded by a nucleic acid according to the invention. Examples of such a protein or fragment are ~~for example~~ proteins described in ~~for example Table 2 of the description, for example~~ Table 2. For example, a cpsA protein is provided that functionally ~~encoding~~ encodes regulation of capsular polysaccharide synthesis, whereas cpsB and cpsC are functionally involved in ~~chain-in-chain~~ chain-in-chain length determination. Other proteins or functional fragments ~~thereof~~ thereof, as provided by the invention, such as cpsD, E, F, G, H, I, J, K and related proteins, are involved in polysaccharide biosynthesis, ~~functioning for example~~ functioning, for example, as glucosyl- or glycosyltransferase in polysaccharide biosynthesis of *Streptococcus suis* capsular antigen.

[0041] The invention ~~furthermore~~ also provides a method ~~to produce~~ of producing a *Streptococcus suis* capsular antigen ~~comprising~~ including using a protein or functional fragment

thereof as provided by the invention, and provides therewith a *Streptococcus suis* capsular antigen obtainable by such a method.

[0042] A comparison of the predicted amino acid sequences of the *cps2* genes with sequences found in the databases allowed the assignment of functions to the open reading frames. The central region contains the ~~type-specific~~ type-specific glycosyltransferases and the putative polysaccharide polymerase. This region is flanked by two regions encoding for proteins with common functions, such as regulation and transport of polysaccharide across the membrane. Biosynthesis of *Streptococcus* capsular polysaccharide antigen using a protein or functional fragment thereof is advantageously used in chemo-enzymatic synthesis and the development of vaccines which offer protection against serotype-specific Streptococcal disease, and is also advantageously used in the synthesis and development of multivalent vaccines against Streptococcal infections. Such vaccines elicit anticapsular antibodies which confer protection.

[0043] Furthermore, the invention provides an acapsular *Streptococcus* mutant for use in a vaccine, a vaccine strain derived thereof and a vaccine derived thereof. Surprisingly, and against the grain of common doctrine, the invention provides use of a *Streptococcus* mutant deficient in capsular expression in a vaccine.

[0044] Acapsular *Streptococcus* mutants have long been known in the art and can be found in nature. Griffith (~~J. Hyg.~~ J. Hyg. 27:113-159, 1928) demonstrated that pneumococci could be transformed from one type to another. If he injected live rough (acapsular or unencapsulated) type 2 pneumococci into mice, the mice would survive. If, however, he injected the same dose of live rough type 2 mixed with heat-killed smooth (encapsulated) type 1 into a mouse, the mouse would die, ~~and~~ and, from the ~~blood~~ blood, he could isolate live smooth type 1 pneumococci. At that time, the significance of this transforming principle was not understood. However, understanding came when it was shown that DNA constituted the genetic material responsible for phenotypic changes during transformation.

[0045] *Streptococcus* mutants deficient in capsular expression are found in several forms. Some are fully deficient and have no capsule at all, others form a deficient capsule, ~~characterised~~ characterized by a mutation in a capsular gene cluster. Deficiency ~~can for instance~~ can, for instance, include capsular formation wherein the organization of the capsular material has been ~~re-arranged~~, as

~~for example demonstrable~~ rearranged as, for example, demonstrable by electron microscopy. Yet others have a nearly fully developed capsule which is only deficient in a particular sugar component.

[0046] Now, after much advance of biotechnology and despite the fact that little is still known about the exact ~~localisation~~ localization and sequence of genes involved in capsular synthesis in Streptococci, it is possible to create mutants of Streptococci, ~~for example~~ example, by homologous recombination or transposon mutagenesis, which ~~has for example~~ has, for example, been done for GAS (Wessels et al., ~~PNAS~~ PNAS 88:8317-8321, 1991), for GBS (~~Wesels~~ (Wessels et al., ~~PNAS~~ PNAS 86: 8983-8987, 1989), for *S. suis* (Smith, ID-DLO Annual report 1996, page 18-19; Charland et al., ~~Microbiol.~~ Microbiol. 144:325-332, 1998) ~~and for~~ and *S. pneumonia pneumoniae* (Kolkman et al., J. Bact. 178:3736-3741, 1996). Such recombinant derived mutants, or isogenic mutants, can easily be compared with the wild-type strains from which they have been derived.

[0047] In a preferred embodiment, the invention provides use of a recombinant-derived *Streptococcus* mutant deficient in capsular expression in a vaccine. Recombinant techniques useful in producing such mutants ~~are for example~~ are, for example, homologous recombination, transposon ~~mutagenises,~~ mutagenesis, and others, ~~whereby~~ wherein deletions, insertions or ~~(point) mutations~~ (point) mutations are introduced in the genome. Advantages of using recombinant techniques ~~are~~ include the stability of the obtained mutants (especially with homologous recombination and double cross-over techniques), and the knowledge about the exact site of the deletion, mutation or insertion.

[0048] ~~In a much preferred~~ another embodiment, the invention provides a stable mutant deficient in capsular expression ~~obtainable~~ obtained, ~~for example~~ example, through homologous recombination or ~~cross-over~~ cross-over integration events. Examples of such a mutant can be found ~~in the experimental part of this description, for example mutant~~ herein, such as mutants 10cpsB or 10cpsEF ~~is such a~~ are stable ~~mutant~~ mutants as provided by the invention.

[0049] The invention also provides a *Streptococcus* vaccine strain and vaccine that has been derived from a *Streptococcus* mutant deficient in capsular expression. In general, ~~said~~ the strain or vaccine is applicable within the whole range of Streptococcal infections, ~~be it for these with~~ including animals or man or with zoonotic infections. ~~It is of course~~ is, of course, now possible to first select a common vaccine strain and derive a *Streptococcus* mutant deficient in capsular

expression thereof for the selection of a vaccine strain and use in a vaccine according to the invention.

[0050] In a preferred embodiment, the invention provides use of a *Streptococcus* mutant deficient in capsular expression in a vaccine wherein ~~said the~~ *Streptococcus* mutant is selected from the group composed of *Streptococcus* group A, *Streptococcus* group B, *Streptococcus suis* and *Streptococcus pneumoniae pneumoniae*. Herewith the invention provides vaccine strains and vaccines for use with these notoriously heterologous Streptococci, of which a multitude of serotypes exist. With a ~~vaccine~~ vaccine, as provided by the ~~invention~~ invention, that is derived from a specific *Streptococcus* mutant that is deficient in capsular expression, the difficulties relating to lack of heterologous protection can be circumvented since these mutants do ~~not~~ not rely on capsular antigens ~~per se~~ antigens, per se, to induce protection.

[0051] In a preferred embodiment, ~~said the~~ vaccine strain is selected for its ability to ~~survive~~ survive, or even ~~replicate~~ replicate, in an immune-competent host or host cells and thus can persist for a certain period, varying from 1-2 days to more than one or two weeks, in a host, despite its deficient character.

[0052] Although an immunodeficient host will support replication of a wide range of bacteria that are deficient in one or more virulence factors, in ~~general~~ general, it is considered a characteristic of pathogenicity of Streptococci that they can survive for certain periods or replicate in a normal host or host cells such as macrophages. For example, ~~Williams~~ Williams and Blakemore (~~Neuropath. Appl. Neurobiol.~~ (Neuropath. Appl. Neurobiol. 16, 345-356, 1990; ~~Neuropath. Appl. Neurobiol.~~ Neuropath. Appl. Neurobiol. 16, 377-392, 1990; ~~J. Infect. Dis.~~ J. Infect. Dis. 162, 474-481, 1990) show that both polymorphonuclear cells and macrophage cells are capable of phagocytosing pathogenic *S. suis* in pigs lacking anti-*S. suis* ~~antibodies~~ antibodies; only pathogenic bacteria could survive and multiply inside macrophages and the pig.

[0053] In a preferred embodiment, the invention, however, provides a deficient or avirulent mutant or vaccine strain which is capable of surviving at least 4-5 days, preferably at least 8-10 days in ~~said the~~ the host, thereby allowing the development of a solid immune response to subsequent *Streptococcus* infection,

[0054] Due to its persistent but avirulent character, a *Streptococcus* mutant or vaccine strain, as provided by the ~~invention~~ invention, is well suited to generate specific and/or long-lasting immune responses against Streptococcal ~~antigens, moreover because~~ antigens. Moreover, possible specific immune responses of the host directed against a capsule are relatively irrelevant because a vaccine strain, as provided by the ~~invention~~ invention, is ~~in general typically not recognised~~ recognized by such antibodies.

[0055] In addition, the invention provides a *Streptococcus* vaccine strain, according to the ~~invention~~ invention, which strain ~~comprises~~ includes a mutant capable of expressing a *Streptococcus* virulence factor or antigenic determinant.

[0056] In a preferred embodiment, the invention provides a *Streptococcus* vaccine strain, according to the ~~invention~~ invention, which strain ~~comprises~~ includes a mutant capable of expressing a *Streptococcus* virulence factor wherein ~~said~~ the virulence factor or antigenic determinant is selected from a group of cellular components, such as muramidase-released protein (MRP) (“MRP”), extracellular factor (EF) (“EF”), and cell-membrane associated proteins, 60kDA heat shock protein, pneumococcal surface protein A (Psp A), pneumolysin, C protein, protein M, fimbriae, ~~haemagglutinins and haemolysin~~ hemagglutinins and hemolysis or components functionally related thereto.

[0057] In a ~~much~~ preferred embodiment, the invention provides a *Streptococcus* vaccine strain ~~according to the invention which strain comprises~~ including a mutant capable of over-expressing ~~said~~ the virulence factor. In this way, the invention provides a vaccine strain for incorporation in a vaccine which specifically causes a host to ~~provide a~~ immune response directed against antigenically important determinants of virulence (listed above), thereby providing specific protection ~~directed against said~~ the determinants. Over-expression ~~can for example~~ can, for example, be achieved by cloning the gene involved behind a strong promoter, which ~~is for example~~ is, for example, constitutionally expressed in a multicopy system, either in a ~~plasmid~~ plasmid or via ~~integration~~ integration in a genome.

[0058] In yet another embodiment, the invention provides a *Streptococcus* vaccine strain, according to the ~~invention which comprises~~ invention, including a mutant capable of

expressing a non-*Streptococcus* protein. Such a vector-*Streptococcus* vaccine strain allows, when used in a vaccine, protection against ~~other pathogens other than *Streptococcus*~~ *Streptococcus*.

[0059] Due to its persistent but avirulent character, a *Streptococcus* vaccine strain or mutant as provided by the invention is well suited to generate specific and long-lasting immune responses, not only against Streptococcal antigens, but also against other antigens ~~when these are expressed by said the strain~~. ~~Especially~~ Specifically, antigens derived from another pathogen are now expressed without the detrimental effects of ~~said the antigen or pathogen~~ which would otherwise have harmed the host.

[0060] An example of such a vector is a *Streptococcus* vaccine strain or mutant wherein ~~said the~~ antigen is derived from a pathogen, such as *Actinobacillus pleuropneumonia*, *Mycoplasmatae*, *Bordetella*, *pasteurella*, *E. coli*, *Salmonella*, *campylobacter*, *Serpulina* and others.

[0061] The invention also provides a vaccine ~~comprising including a~~ *Streptococcus* vaccine strain or mutant according to the invention and ~~further comprising a~~ pharmaceutically acceptable carrier or adjuvant. Carriers or adjuvants are well known in the ~~art~~, art; examples are phosphate buffered saline, physiological salt solutions, (double-) ~~oil in water emulsions~~, oil-in-water emulsions, aluminumhydroxide, Specol, block- or co-polymers, and others.

[0062] A vaccine according to the invention can ~~comprise include~~ a vaccine strain either in a killed or live form. For example, a killed vaccine ~~comprising including a~~ strain having (over)expressed a Streptococcal or heterologous antigen or virulence factor is very well suited for eliciting an immune response. In a preferred embodiment, the invention provides a vaccine wherein ~~said the strain is live, due to its persistent but avirulent character~~, character; a *Streptococcus* vaccine ~~strain strain~~, as provided by the ~~invention invention~~, is well suited to generate specific and long-lasting immune responses.

[0063] ~~Now that a Streptococcal vaccine is provided by the invention, the~~ The invention also provides a method for controlling or eradicating a Streptococcal disease in a population comprising vaccinating subjects in ~~said the~~ population with a vaccine according to the invention.

[0064] In a preferred embodiment, a method for controlling or eradicating a Streptococcal disease is provided ~~comprising including~~ testing a sample, such as a blood sample, or nasal or throat swab, ~~faeces, feces~~, urine, or other samples such as can be sampled at or after slaughter, collected

from at least-one subject, such as an infant or a pig, in a population partly or ~~wholly~~ wholly vaccinated with a vaccine according to the invention for the presence of encapsulated Streptococcal strains or mutants. Since a vaccine strain or mutant according to the invention is not pathogenic, and can be distinguished from wild-type strains by capsular expression, the detection of (fully) encapsulated Streptococcal strains indicates that wild-type infections are still present. Such wild-type infected subjects can ~~than~~ then be isolated from the remainder of the population until the infection has passed-away. With domestic animals, such as pigs, it is even possible to remove the infected subject from the population as a whole by culling. Detection of wild-type strains can be achieved via traditional culturing techniques, or by rapid detection techniques such as PCR detection.

[0065] In yet another embodiment, the invention provides a method for controlling or eradicating a Streptococcal disease ~~comprising~~ including testing a sample collected from at least one subject in a population partly or wholly vaccinated with a vaccine according to the invention for the presence of capsule-specific antibodies directed against Streptococcal strains. ~~Capsule-specific~~ Capsule-specific antibodies can be detected with classical techniques known in the art, such as used for Lancefield's group typing or serotyping.

[0066] A ~~much~~-preferred embodiment of a ~~method provided by the invention~~ for controlling or eradicating a Streptococcal disease in a population ~~comprises~~ includes vaccinating subjects in ~~said~~ the population with a vaccine according to the invention and testing a sample collected from at least one subject in ~~said~~ the population for the presence of encapsulated Streptococcal strains and/or for the presence of capsule-specific antibodies directed against Streptococcal strains.

[0067] For example, a method is provided ~~according to the invention~~ wherein ~~said~~ the Streptococcal disease is caused by *Streptococcus suis*.

[0068] The invention also provides a diagnostic assay for testing a sample for use in a method according to the invention ~~comprising~~ including at least one means for the detection of encapsulated Streptococcal strains and/or for the detection of capsule-specific antibodies directed against Streptococcal strains.

[0069] The invention ~~furthermore~~ further provides a vaccine ~~comprising~~ including an antigen according to the invention and ~~further comprising~~ a suitable carrier or adjuvant. The

immunogenicity of a capsular antigen provided by the invention ~~is for example~~ is, for example, increased by linking to a carrier (such as a carrier protein), allowing the recruitment of T-cell help in developing an immune response.

[0070] The invention further provides a recombinant ~~micro-organism~~ microorganism provided with at least a part of a capsular gene cluster derived from *Streptococcus suis*. The invention provides for example a lactic acid bacterium provided with at least a part of a capsular gene cluster derived from *Streptococcus suis*. Various food-grade lactic acid bacteria (~~Lactococcus lactis, Lactobacillus casei, Lactobacillus plantarium and Streptococcus gordonii~~) (Lactococcus lactis, Lactobacillus casei, Lactobacillus plantarium and Streptococcus gordonii) have been used as delivery systems for mucosal immunization. It has now been shown that oral (or mucosal) administration of recombinant ~~L. lactis, Lactobacillus, and Streptococcus gordonii~~ L. lactis, Lactobacillus, and Streptococcus gordonii can elicit local IgA and/or IgG antibody responses to an expressed antigen. The use of oral routes for immunization against infective diseases is desirable because oral vaccines are easier to ~~administer~~, administer and have higher compliance rates, and because mucosal surfaces are the portals of entry for many pathogenic microbial agents. It is within the skill of the artisan to provide such ~~micro-organisms~~ microorganisms with (additional) genes.

[0071] The invention further provides a recombinant *Streptococcus suis* mutant provided with a modified capsular gene cluster. It is within the skill of the artisan to swap genes within a species. In a preferred embodiment, an avirulent *Streptococcus suis* mutant is selected to be provided with at least a part of a modified capsular gene cluster according to the invention.

[0072] The invention further provides a vaccine ~~comprising a micro-organism~~ including a microorganism or a mutant provided by the invention. An advantage of such a vaccine over currently used vaccines is that they ~~comprise~~ include accurately defined ~~micro-organisms~~ microorganisms and ~~well-characterised~~ well-characterized antigens, allowing accurate determination of immune responses against various antigens of choice.

[0073] The invention is further explained in the experimental part of this description without limiting the invention thereto.

~~Description of the Figures~~ DESCRIPTION OF THE DRAWINGS

[0074] **FIG. 1** illustrates the organization of the *cps2* gene cluster of *S. suis* type 2.

[0075] (A) Genetic map of the *cps2* gene cluster. The shadowed arrows represent potential ORFs. Interrupted ORFs indicate the presence of stop codons or frame-shift mutations. Gene designations are indicated below the ORFs. The closed arrows indicate the position of the potential promoter sequences. | indicates the position of the potential transcription regulator sequence. ||| indicates the position of the 100-bp repeated sequence.

[0076] (B) Physical map of the *cps2* locus. Restriction sites are as follows: A: *AluI*; C: *ClaI*; ~~E~~, E: *EcoRI*; H, H: *HindIII*; ~~K~~, K: *KpnI*; ~~M~~, M: *MluI*; ~~N~~, N: *NsiI*; ~~P~~, P: *PstI*; ~~S~~, S: *SnaBI*; Sa: *SacI*; ~~X~~, X: *XbaI*.

[0077] (C) The DNA fragments cloned in the various plasmids.

[0078] **FIG. 2** illustrates ethidium bromide stained agarose gel showing PCR products obtained with chromosomal DNA of *S. suis* strains belonging to the serotypes 1, 2, 1/2, 9 and 14 and *cps2J*, *cps1I*, and *cps9H* primer sets as described herein.

[0079] (A) *cps1I* primers; (B) *cps2J* primers and (C) *cps9H* primers.

[0080] Lanes 1-3: serotype 1 strains; lanes 4-6: serotype 2 strains; lanes 7-9: serotype 1/2 strains; lanes 10-12: serotype 9 strains and lanes 13-15: serotype 14 strains.

[0081] (B) Ethidium bromide stained agarose gel showing PCR products obtained with tonsillar swabs collected from pigs carrying *S. suis* type 2, type 1 or type 9 strains and *cps2J*, *cps1I* and *cpsH* primer sets as described in Materials and Methods. Bacterial DNA suitable for PCR was prepared by using the multiscreen methods as described previously (20).

[0082] ~~(A) *cpsH*~~ (C) *cps1I* primers. (B) *cps2J* primers and (C) *cps9H* primers.

[0083] Lanes 1-3: PCR products obtained with tonsillar swabs collected from pigs carrying *S. suis* type 1 strains; lanes 4-6: PCR products obtained with tonsillar swabs collected from pigs carrying ~~*S. suis*~~ *S. suis* type 2 strains; lanes 7-9: PCR ~~products~~ products obtained with tonsillar swabs collected from pigs carrying *S. suis* type 9 strains; lanes 10-12: PCR ~~products~~ products obtained with chromosomal DNA from serotype 9, 2 and 1 strains respectively; lane 13: negative control, no DNA present.

[0084] FIG. 3 illustrates the CPS2 nucleotide sequences and corresponding amino acid sequences from the open reading frames.

[0085] FIG. 4 illustrates the CPS1 nucleotide sequences and corresponding amino acid sequences from the open reading frames.

[0086] FIG. 5 illustrates the CPS9 nucleotide sequences and corresponding amino acid sequences from the open reading frames.

[0087] FIG. 6 illustrates the CPS7 nucleotide sequences and corresponding amino acid sequences from the open reading frames.

[0088] FIG. 7 illustrates alignment of the N-terminal parts of Cps2J and Cps2K.

[0089] Identical amino acids are marked by bars. The amino acids shown in bold are also conserved in ~~CPS14I~~ ~~Cps14J~~ Cps14I, Cps24J of *S. pneumoniae* and several other glycosyltransferases (19). The aspartate residues marked by asterisks are strongly conserved.

[0090] FIG. 8 illustrates transmission electron micrographs of thin sections of various *S. suis* strains.

[0091] (A) ~~wild-type~~ wild-type strain 10;

[0092] (B) mutant strain 10cpsB;

[0093] (C) mutant strain 10cpsEF.

[0094] Bar = 100 nm

[0095] FIG. 9 illustrates the kinetics of phagocytosis of ~~wild-type~~ wild-type and mutant *S. suis* strains.

[0096] (A) Kinetics of phagocytosis of ~~wild-type~~ wild-type and mutant *S. suis* strains by porcine ~~alveolar~~ alveolar macrophages. Phagocytosis was determined as described herein. The Y-axis represents the number of CFU per milliliter in the supernatant fluids as determined by plate counting, the X-axis represents time in minutes.

[0097] □ ~~wild-type~~ wild-type strain 10;

[0098] ○ mutant strain 10cpsB;

[0099] Δ mutant strain 10cpsEF.

[00100] (B) Kinetics of intracellular killing of ~~wild-type~~ wild-type and mutant *S. suis* strains by porcine AM. The intracellular killing was determined as described herein. The Y-axis

represents the number of CFU per ml in the supernatant fluids after lysis of the macrophages as determined by plate counting, the X-axis represents time in minutes.

[00101] □ ~~wild-type~~ wild-type strain 10;

[00102] o mutant strain 10cpsB;

[00103] Δ mutant strain 10cpsEF.

[00104] **FIG. 10** illustrates the nucleotide sequence alignment of the highly conserved 100-bp repeated element.

[00105] 1) 100-bp repeat between cps2G and cps2H

[00106] 2) 100-bp repeat within “cps2M”

[00107] 3) 100-bp repeat between cps20 and cps2P

[00108] **FIG. 11** illustrates the cps2, cps9 and cps7 gene clusters of *S. suis* serotypes 2, 9 and 7.

[00109] (A) Genetic organization of the cps2 gene cluster [84]. The large arrows represent potential ORFs. Gene designations are indicated below the ORFs. Identically filled arrows represent ORFs which showed homology. The small closed arrows indicate the position of the potential promoter sequences. | indicates the position of the potential transcription regulator sequence.

[00110] (B) Physical map and genetic organization of the cps9 gene cluster [15]. Restriction sites are as follows: B: *Bam*HI; P: *Pst*I; H: *Hind*III; X: *Xba*I. The DNA fragments cloned in the various plasmids are indicated. The open arrows represent potential ORFs.

[00111] (C) Physical map and genetic organization of the ~~cps7 gene~~ cps7 gene cluster. Restriction sites are as follows: C: *Clal*; P: *Pst*I; Sc: *Sca*I. The DNA fragments cloned in the various plasmids are indicated. The open arrows represent potential ~~ORFs~~ ORFs.

[00112] **FIG. 12** illustrates ~~Ethidium~~ ethidium bromide stained agarose gel showing PCR products.

[00113] (A) Ethidium bromide stained agarose gel showing PCR products obtained with chromosomal DNA of *S. suis* strains belonging to the serotypes 1, 2, 9 and 7 and the cps7H primer set. Strain designations are indicated above the lanes. C: negative control, no DNA present. M: molecular size marker (lambda digested with *Eco*RI and *Hind*III).

[00114] (B) Ethidium bromide stained agarose gel showing PCR products obtained with serotype 7 strains collected in different countries and from different organs. Bacterial DNA suitable for PCR was prepared by using the multiscreen method as described herein [89]. Strain designations are indicated above the lanes. M: molecular size marker (lambda digested with *EcoRI* and *HindIII*).

DETAILED DESCRIPTION OF THE INVENTION

Experimental part

MATERIAL AND METHODS

Bacterial strains and growth conditions.

[00115] The bacterial strains and plasmids used in this study are listed in Table 1. *S. suis* strains were grown in Todd-Hewitt broth (code CM189, Oxoid), and plated on Columbia agar blood base (code CM331, Oxoid) containing 6% (v/v) horse blood. *E. coli* strains were grown in Luria broth (28) and plated on Luria broth containing 1.5% (w/v) agar. If required, antibiotics were added to the plates at the following concentrations: spectinomycin: 100 µg/ml for *S. suis* and 50 µg/ml for *E. coli* and ampicillin, 50 µg/ml.

[00116] **Serotyping.** The *S. suis* strains were ~~serotypes~~ serotyped by the slide agglutination test with serotype-specific antibodies (44).

[00117] **DNA techniques.** Routine DNA manipulations were performed as described by Sambrook et al. (36).

[00118] **Alkaline phosphatase activity.** To screen for PhoA fusions in *E. coli*, plasmid libraries were constructed. Therefore, chromosomal DNA of *S. suis* type 2 was digested with *AluI*. The 300-500-bp fragments were ligated to *SmaI*-digested pPHOS2. Ligation mixtures were transformed to the PhoA⁻ *E. coli* strain CC118. Transformants were plated on LB media supplemented with 5-Bromo-4-chloro-3-indolylfosfaat (BCIP, 50 µg/ml, Boehringer, Mannheim, Germany). Blue colonies were purified on fresh LB/BCIP plates to verify the blue phenotype.

[00119] **DNA sequence analysis.** DNA sequences were determined on a 373A DNA Sequencing System (Applied Biosystems, Warrington, GB). Samples were prepared by ~~use of a~~ using an ABI/PRISM dye terminator cycle sequencing ready reaction kit (Applied Biosystems). Sequencing data were assembled and analyzed using the MacMollyTetra program. Custom-made

sequencing primers were purchased from Life Technologies. Hydrophobic stretches within proteins were predicted by the method of Klein et al. (17). The BLAST program available on Netscape Navigator™ was used to search for protein sequences related to the deduced amino acid sequences.

[00120] Construction of gene-specific knock-out mutants of *S. suis*. To construct the mutant strains 10cpsB and ~~10cpsEF~~ 10cpsEF, we electrotransformed the pathogenic serotype 2 strain 10 (45, 49) of *S. suis* with pCPS11 and pCPS28 respectively. In these ~~plasmids~~ plasmids, the *cpsB* and *cpsEF* genes were disturbed by the insertion of a spectinomycin-resistance gene. To create ~~pCPS11~~ pCPS11, the internal 400 bp *PstI*-*Bam*HI fragment of the *cpsB* gene in pCPS7 was replaced by the Spc^R gene. For this ~~purpose~~ purpose, pCPS7 was digested with *PstI* and *Bam*HI and ligated to the 1,200-bp *PstI*-*Bam*HI fragment, containing the Spc^R ~~gen,~~ gene, from pIC-spc. To construct ~~pCPS28~~ pCPS28, we have used pIC20R. In this plasmid we inserted the *KpnI*-*SalI* fragment from pCPS17 (resulting in pCPS25) and the *XbaI*-*ClaI* fragment from pCPS20 (resulting in pCPS27). pCPS27 was digested with *PstI* and *XhoI* and ligated to the 1,200-bp *PstI*-*XhoI* fragment, containing the Spc^R gene of pIC-spc. The electrotransformation to *S. suis* was carried out as described before (38).

[00121] Southern blotting and hybridization. Chromosomal DNA was isolated as described by Sambrook et al. (36). DNA fragments were separated on 0.8% agarose gels and transferred to Zeta-Probe GT membranes (Bio-Rad) as described by Sambrook et al. (36). DNA probes were ~~labelled~~ labeled with [(-³²P)dCTP (3000 Ci mmol⁻¹ Amersham) by use of a random primed ~~labelling~~ labeling kit (Boehringer). The DNA on the blots was hybridized at 65°C with appropriate DNA probes as recommended by the supplier of the Zeta-Probe membranes. After hybridization, the membranes were washed twice with a solution of 40 mM sodium phosphate, pH 7.2, 1 mM EDTA, 5% SDS for 30 min at 65°C and twice with a solution of 40 mM sodium phosphate, pH 7.2, 1 mM EDTA, 1% SDS for 30 min at 65°C.

[00122] PCR. The primers used in the *cps2J* PCR correspond to the positions 13791-13813 and 14465-14443 in the *S. suis cps2* locus. The sequences were: 5'-CAAACGCAAGGAATTACGGTATC-3' (SEQ ID NO:1) and 5'-GAGTATCTAAAGAATGCCTATTG-3' (SEQ ID NO:2). The primers used for the *cps1I* PCR correspond to the positions 4398-4417 and 4839-4821 in the *S. suis cps1* sequence. The sequences

were: 5'-GGCGGTCTAGCAGATGCTCG-3' (SEQ ID NO:3) and 5'-GCGAACTGTTAGCAATGAC-3' (SEQ ID NO:4). The primers used in the *cps9H* PCR correspond to the positions 4406-4126 and 4494-4475 in the *S. suis cps9* sequence. The sequences were: 5'-GGCTACATATAATGGAAGCCC3' (SEQ ID NO:5) and 5'-CGGAAGTATCTGGGCTACTG-3' (SEQ ID NO:6).

[00123] Construction of gene-specific knock-out mutants of *S. suis*. To construct the mutant strains 10cpsB and ~~10cpsEF~~ 10cpsEF, we electrotransformed the pathogenic serotype 2 strain 10 of *S. suis* with pCPS11 and pCPS28 respectively. In these ~~plasmids~~ plasmids, the *cpsB* and *cpsEF* genes were disturbed by the insertion of a spectinomycin-resistance gene. To create ~~pCPS11~~ pCPS11, the internal 400 bp *PstI*-*Bam*HI fragment of the *cpsB* gene in pCPS7 was replaced by the Spc^R gene. For this ~~purpose~~ purpose, pCPS7 was digested with *PstI* and *Bam*HI and ligated to the 1,200-bp *PstI*-*Bam*HI fragment, containing the Spc^R ~~gen,~~ gene, from pIC-spc. To construct ~~pCPS28~~ pCPS28, we have used pIC20R. In this ~~plasmid~~ plasmid, we inserted the *KpnI*-*SalI* fragment from pCPS17 (resulting in pCPS25) and the *XbaI*-*ClaI* fragment from pCPS20 (resulting in pCPS27). pCPS27 was digested with *PstI* and *XhoI* and ligated to the 1,200-bp *PstI*-*XhoI* fragment, containing the spc^R gene of pIC-Spc. The electrotransformation to *S. suis* was carried out as described before (38).

[00124] Phagocytosis assay. Phagocytosis assays were performed as described by Leij-~~et al.~~ et al. (23). Briefly, to opsonize the cells, 10⁷ *S. suis* cells were incubated with 6% SPF-pig serum for 30 min at 37°C in a head-over-head rotor at 6 rpm. 10⁷ AM and 10⁷ opsonized *S. suis* cells were combined and incubated at 37°C under continuous rotation at 6 rpm. At 0, 30, 60 and 90 min, 1-ml samples were collected and mixed with 4 ml of ice-cold EMEM to stop phagocytosis. Phagocytes were removed by centrifugation for 4 min at 110 x g and 4°C. The number of ~~colony-forming~~ colony-forming units, (CFU) (“CFU”) in the supernatants was determined. Control experiments were carried out simultaneously by combining 10⁷ opsonized *S. suis* cells with EMEM (without AM).

[00125] Killing assays. AM (10⁷/ml) and opsonized *S. suis* cells (10⁷/ml) were mixed 1 : 1 and incubated for 10 min at 37°C under continuous rotation at 6 rpm. Ice-cold EMEM was added to stop further phagocytosis and killing. To remove extracellular *S. suis* cells, phagocytes were washed

twice (4 min, 110 x g, 4°C) and resuspended in 5 ml EMEM containing 6% SPF serum. The tubes were incubated at 37°C under rotation at 6 rpm. After 0, 15, 30, 60 and 90 min, samples were collected and mixed with ice-cold EMEM to stop further killing. The samples were centrifuged for 4 min at 110 x g at 4°C and the phagocytic cells were lysed in EMEM containing 1% saponine for 20 min at room temperature. The number of CFU in the suspensions was determined.

[00126] Pigs. Germfree pigs, ~~cross-breeds~~ crossbreeds of Great Yorkshire and Dutch ~~landrace~~, Landrace, were obtained from sows by caesarian sections. The surgery was performed in sterile flexible film isolators. Pigs were allotted to groups, each consisting of 4 pigs, and were housed in sterile stainless steel incubators.

[00127] Experimental infections. Pigs were inoculated intranasally with *S. suis* type 2 as described before. To predispose the pigs for infection with *S. suis*, five-day old pigs were inoculated intranasally with about 10^7 CFU of *Bordetella bronchiseptica* strain 92932. Two days ~~later~~ later, the pigs were inoculated intranasally with *S. suis* type 2 (10^6 CFU). Pigs were monitored twice daily for clinical signs of disease, such as fever, nervous signs and lameness. Blood samples were collected three times a week from each pig. White blood cells were counted with a cell counter. To monitor infection with *S. suis* and *B. bronchiseptica* and to check for absence of contaminants, we collected swabs of nasopharynx and feces daily. The swabs were plated directly onto Columbia agar containing 6% horse blood. After ~~three-weeks~~ weeks, the pigs were killed and examined for pathological changes. Tissue specimens from the central nervous system, serosae, and joints were examined bacteriologically and histologically as described ~~before~~ herein (45, 49). Colonization of the serosae was scored positively when *S. suis* was isolated from the pericardium, thoracic pleura or the peritoneum. Colonization of the joints was scored positively when *S. suis* was isolated from one or more joints (12 joints per animal were scored).

[00128] Vaccination and challenge. One week old pigs were vaccinated intravenously with a dosage of 10^6 cfu of the ~~S. suis~~ *S. suis* strains 10cpsEF or 10cpsB. Three weeks ~~later~~ later, the pigs were challenged intravenously with the pathogenic serotype 2 strain 10 (10^7 cfu). Disease monitoring, ~~haematological~~, hematological, serological and bacteriological examinations as well as post-mortum examinations were as described before under experimental infections.

[00129] Electron Microscopy. Bacteria were prepared for electron microscopy as described by Wagenaar et al. (50). Shortly, bacteria were mixed with agarose MP (Boehringer) of 37°C to a concentration of 0.7%. The mixture was immediately cooled on ice. Upon gelifying, samples were cut into 1 to 1.5 mm slices and incubated in a fixative containing 0.8% glutaraldehyde and 0.8% osmiumtetroxide. Subsequently, the samples were fixed and stained with uranyl acetate by microwave stimulation, dehydrated and imbedded in eponaraldite resin. Ultra-thin sections were counterstained with lead citrate and examined with a Philips CM 10 electron microscope at 80 kV. (FIG. 8.)

[00130] Isolation of porcine alveolar macrophages (AM). Porcine AM were obtained from the lungs of ~~specific-pathogen-free (SPF)~~ pathogen-free ("SPF") pigs. Lung lavage samples were collected as described by van Leengoed ~~et al.~~ et al. (43). Cells were suspended in EMEM containing 6% (v/v). SPF-pig serum and adjusted to 10 cells per ml.

RESULTS

Identification of the *cps* locus.

[00131] The *cps* locus of *S. suis* type 2 was identified ~~by making use of~~ through a strategy developed for the genetic identification of exported proteins (13, 31). In this ~~system we made use of~~ system, a plasmid (pPHOS2) containing a truncated alkaline phosphatase gene (13) was used. The gene lacked the promoter sequence, the translational start site and the signal sequence. The truncated gene is preceded by a unique *Sma*I restriction site. Chromosomal DNA of *S. suis* type 2, digested with *Alu*I, was randomly cloned in this restriction site. Because translocation of PhoA across the cytoplasmic membrane of *E. coli* is required for enzymatic activity, the system can be used to select for *S. suis* fragments containing a promoter sequence, a translational start site and a functional signal sequence. Among 560 individual *E. coli* clones tested, 16 displayed a dark blue phenotype when plated on media containing BCIP. DNA sequence analysis of the inserts from several of these plasmids ~~were~~ was performed (results not shown) and the deduced amino acid sequences were analyzed. The hydrophobicity profile of one of the clones (pPHOS7, results not shown) showed that the N-terminal part of the sequence resembled the characteristics of a typical signal peptide: a short hydrophilic N-terminal region is followed by a hydrophobic region of 38 amino acids. These data

indicate that the *phoA* system was successfully used for the selection of *S. suis* genes encoding exported proteins. Moreover, the sequences were analyzed for similarities present in the databases. The sequence of pPHOS7 showed a high similarity (37% identity) with the protein encoded by the *cps14C* gene of *Streptococcus pneumoniae* (19). This strongly suggests that pPHOS7 contains a part of the *cps* operon of *S. suis* type 2.

[00132] Cloning of the flanking *cpa* genes. In order to clone the flanking *cps* genes of *S. suis* ~~type 2~~ type 2, the insert of pPHOS7 was used as a probe to identify chromosomal DNA fragments which contain flanking *cps* genes. A 6-kb *Hind*III fragment was identified and cloned in pKUN19. This yielded clone pCPS6 (~~Fig. 1C~~) (FIG. 1, part C). Sequence analysis of the insert of pCPS6 revealed that pCPS6 most probably contained the 5'-end of the *cps* locus, but still lacked the 3'-end. Therefore, sequences of the 3'-end of pCPS6 were in turn used as a probe to identify chromosomal fragments containing *cps* sequences located further downstream. These fragments were also cloned in pKUN19, resulting in pCPS17. Using the same system of chromosomal ~~walking~~ walking ~~we subsequently generated the plasmid~~ walking, plasmids pCPS18, pCPS20, pCPS23 and pCPS26, containing downstream *cps* sequences were subsequently generated.

[00133] Analysis of the *cps* operon. The complete nucleotide sequence of the cloned fragments was determined (~~figure 4~~) (FIG. 4). Examination of the compiled sequence revealed the presence of at least 13 potential open reading ~~frame~~ frames (Orfs), which were designated as Orf 2Y, Orf2X and Cps2A-Cps2K (~~Fig. 1A~~) (FIG. 1, part A; FIG. 11, part A). Moreover, a 14th, ~~incomplete,~~ incomplete Orf (Orf 2Z) was located at the 5'-end of the sequence. Two potential promoter sequences were identified. One was located 313 bp (locations 1885-1865 and 1884-1889) upstream of Orf2X. The other potential promoter sequence was located 68 bp upstream of Orf2Y (locations 2241-2236 and 2216-2211). Orf2Y is expressed in opposite orientation. Between Orfs 2Y and ~~2Z~~ 2Z, the sequence contained a potential stem-loop structure, which could act as a transcription terminator. Each Orf is preceded by a ribosome-binding site and the majority of the Orfs are very closely linked. The only significant intergenic gap was found between Cps2G and Cps2H (389 nucleotides). However, no obvious promoter sequences or potential stem-loop structures were found in this region. These data suggest that Orf2X and Cps2A-Cps2K are arranged as an operon.

[00134] An overview of all Orfs with their properties is shown in Table 2. The majority of the predicted gene products is related to proteins involved in polysaccharide biosynthesis. Orf2Z showed some similarity with the YitS protein of *Bacillus subtilis*. YitS was identified during the sequence analysis of the complete genome of *B. subtilis*. The function of the protein is unknown.

[00135] Orf2Y showed similarity with the YcxD protein of *B. subtilis* (53). Based on the similarity between YcxD and MocR of *Rhizobium meliloti* (33), YcxD was suggested to be a regulatory protein.

[00136] Orf2X showed similarity with the hypothetical YAAA proteins of *Haemophilus influenzae* and *E. coli*. The function of these proteins is unknown.

[00137] The gene products encoded by the *cps2A*, *cps2B*, *cps2C* and *cps2D* genes showed approximate similarity ~~with~~ to the CpsA, CpsC, CpsD and CpsB proteins of several serotypes of *Streptococcus pneumoniae* (19), respectively. This ~~suggest~~ suggests similar functions for these proteins. Hence, Cps2A may have a role in the regulation of the capsular polysaccharide synthesis. Cps2B and Cps2C could be involved in the chain length determination of the type 2 capsule and Cps2C can play an additional role in the export of the polysaccharide. The Cps2D protein of *S. suis* is related to the CpsB protein of *S. pneumoniae* and to proteins encoded by genes of several other Gram-positive bacteria involved in polysaccharide or exopolysaccharide synthesis, but their function is unknown (19).

[00138] The protein encoded by the *cps2E* gene showed similarity to several bacterial proteins ~~with-glycosyl-transferase~~ glycosyltransferase activities: Cps14E and Cps19fE of *S. pneumoniae* serotypes 14 and 19F (18, 19, 29), CpsE of *Streptococcus salvarius* (X94980) and CpsD of *Streptococcus agalactiae* (34). Recently, Kolkman ~~et al.~~ et al. (18) showed that Cps14E is a glucosyl-1-phosphate transferase that links glucose to a lipid carrier, the first step in the biosynthesis of the *S. pneumoniae* type 14 repeating unit. Based on these ~~data~~ data, a similar function may be fulfilled by Cps2E of *S. ~~suis~~ suis*.

[00139] The protein encoded by the *cps2F* gene showed similarity to the protein encoded by the *rfbU* gene of *Salmonella enteritica* (25). This similarity is most pronounced in the C-terminal regions of these proteins. The *rfbU* gene was shown to ~~encoded~~ encode mannosyltransferase activity (25).

[00140] The *cps2G* gene encoded a protein that showed moderate similarity with the *rfbF* gene product of *Campylobacter hyoilei* (22), the *epsF* gene product of *S. thermophilus* (40) and the *capM* gene product of *S. aureus* (24). On the basis of ~~similarity~~ similarity, the *rfbF*, *epsF* and *capM* genes are suggested to ~~encoded~~ encode galactosyltransferase activities. Hence, a similar ~~glycosyl transferase~~ glycosyltransferase activity could be fulfilled by the *cps2G* gene product.

[00141] The *cps2H* gene encodes a protein that is similar to the N-terminal region of the *lgtD* gene product of *Haemophilus influenzae* (U32768). Moreover, the hydrophobicity plots of Cps2H and LgtD looked very similar in these regions (data not shown). Based on sequence ~~similarity~~ similarity, the *lgtD* gene product was suggested to have ~~glycosyl transferase~~ glycosyltransferase activity (U32768).

[00142] The gene product encoded by the *cps2I* gene showed some similarity with a protein of *Actinobacillus actinomycetemcomitans* (AB002668). This protein is part of the gene cluster responsible for the serotype-b-specific antigen of *A. ~~actinomyetemeomitans~~* *actinomycetemcomitans*. The function of the protein is unknown.

[00143] The gene products encoded by the *cps2J* and *cps2K* genes showed significant similarities to the Cps14J protein of *S. pneumoniae*. The *cps14J* gene of *S. pneumoniae* was shown to encode a β -1,4-galactosyltransferase activity. In *S. ~~pneumoniae pneumoniae~~* *pneumoniae*, CpsJ is responsible for the addition of the fourth ~~(i.e. (i.e.~~ last) sugar in the synthesis of the *S. pneumoniae* serotype 14 polysaccharide (20). Even some similarity was found between Cps2J and Cps2K ~~(Fig. (FIG. 2,~~ 25.5% similarity). This similarity was most pronounced in the N-terminal regions of the proteins ~~(FIG. 7)~~ (FIG. 7). Recently, two small conserved regions were identified in the N-terminus of Cps14J and Cps14I and their homologues (20). These regions were predicted to be important for catalytic activity. Both regions, DXS and DXDD ~~(Fig. (FIG. 2)~~ (FIG. 2), were also found in Cps2J and Cps2K.

[00144] **Distribution of the *cps2* genes in other *S. suis* serotypes.** To examine the relationship between the *cps2* genes and *cps* genes in the other *S. suis* serotypes, we performed cross-hybridization experiments. DNA fragments of the individual *cps2* genes were amplified by PCR, ~~labelled~~ labeled with ^{32}P , and used to probe Southern blots of chromosomal DNA of the reference strains of the 35 different *S. suis* serotypes. ~~Large variation~~ variations in the hybridization patterns were observed (Table 4). As a positive ~~control~~ control, we used a probe specific for 16S

rRNA. The 16S rRNA probe hybridized with all serotypes tested. However, none of the other genes tested were common in all serotypes. Based on the genetic organization of the ~~genes~~ genes, it ~~was~~ previously suggested that *orfX* and *cpsA-cpsK* genes are part of one operon and that the ~~protein~~ proteins encoded by these genes are all involved in polysaccharide biosynthesis. OrfY and OrfZ are not a part of this operon, and their role in the polysaccharide biosynthesis is unclear. Based on sequence similarity data, OrfY may be involved in regulation of the *cps2* genes. OrfZ is proposed to be unrelated to polysaccharide biosynthesis. Probes specific for the *orfZ*, *orfY*, *orfX*, *cpsA*, *cpsB*, *cpsC* and *cpsD* genes hybridized with most other serotypes. This suggests that the ~~protein~~ proteins encoded by these genes are not type-specific, but may perform more common functions in biosynthesis of the capsular polysaccharide. This confirms previous data which showed that the *cps2A-cps2D* genes showed strong similarity to *cps* genes of several ~~serotype~~ serotypes of *Streptococcus pneumoniae*. Based on this ~~similarity~~ similarity, Cps2A is possibly a regulatory protein, whereas Cps2B and Cps2C may play a role in length determination and export of polysaccharide. The *cps2E* gene hybridized with DNA of serotypes 1, 2, 14 and 1/2. The *cps2E* gene showed a strong similarity to the *cps14E* gene of *S. pneumoniae* (18). This enzyme was shown to have a glucosyl-1-phosphate activity and catalyzed the transfer of glucose to a lipid carrier (18). These data indicate that a glycosyltransferase closely related to Cps14E may be responsible for the first step in the biosynthesis of polysaccharide in the *S. suis* serotypes 1, 2, 14 and 1/2. The *cps2F*, *cps2G*, *cps2H*, *cps2I* and *cps2J* genes hybridized with chromosomal DNA of serotypes 2 and 1/2 only. The *cps2G* gene showed an additional weak hybridization signal with DNA of serotype 34. In ~~agglutination tests~~ tests, serotype 1/2 showed agglutination with sera specific for serotype 2 as well as with sera specific for serotype 1. This suggests that serotype 1/2 shares antigenic determinants with both types 1 and 2. The hybridization data confirmed these data. All putative glycosyltransferases present in serotype 2 are also present in serotype 1/2. The *cps2K* gene showed a ~~similar~~ similar hybridization pattern ~~as~~ similar to the *cps2E* gene. Hybridization was observed with DNA of serotypes 1, 2, 14 and 1/2. ~~Taken together~~ together, these hybridization data show that the *cps2* gene cluster can be divided ~~in~~ into three regions: a central region containing the type-specific genes is flanked by two regions containing common genes for various serotypes.

[00145] **Cloning of the type-specific *cps* genes of serotypes 1 and 9.** To clone the type-specific *cps* genes of *S. suis* serotype 1 we used 1, the *cps2E* gene was used as a probe to identify chromosomal DNA fragments of type 1 which contain flanking *cps* genes. A 5 kb *EcoRV* fragment was identified and cloned in pKUN19. This yielded pCPS1-1 (~~Fig-1B~~) (FIG. 1, part B). This fragment was in turn used as a probe to identify an overlapping 2.2 kb *HindIII* fragment. pKUN19 containing this *HindIII* fragment was designated pCPS1-2. The same strategy was followed to identify and clone the type-specific *cps* genes of serotype 9. In this case, we used the *cps2D* gene as a probe. A 0.8 kb *HindIII-XbaI* fragment was identified and cloned, yielding pCPS9-1 (~~Fig-1C~~) (FIG. 1, part C). This fragment was in turn used as a probe to identify a 4 kb *XbaI* fragment. pKUN19 containing this 4 kb *XbaI* fragment was designated pCPS9-2.

[00146] **Analysis of the cloned *cps1* genes.** The complete nucleotide sequence of the inserts of pCPS1-1 and pCPS1-2 was determined (~~figure~~) (FIG. 5). Examination of the sequence revealed the presence of five complete and two incomplete Orfs (~~Fig-1B~~) (FIG. 1, part B). Each Orf is preceded by a ribosome-binding site. In accord with data obtained for the *cps2* genes of serotype 2, the majority of the Orfs is very closely linked. The only significant gap (718 bp) was found between Cps1G and Cps1H. No obvious promoter sequences or potential stem-loop structures could be found in this region. This suggests that, as in serotype 2, the *cps* genes in serotype 1 are arranged in an operon.

[00147] An overview of the Orfs and their properties ~~in~~ is shown in Table 2. As expected on the basis of the hybridization data (Table 4), the protein encoded by the *cps1E* gene was related to Cps2E of *S. suis* type 2 (identity of 86%). The fragment cloned in pCPS1-1 lacked the coding region for the first 7 amino acids of the *cps1E* gene.

[00148] The protein encoded by the *cps1F* and *cps1G* genes showed strong similarity to the Cps14F and Cps14G proteins of *Streptococcus pneumoniae* serotype 14, respectively (20). The function of the Cps14F is not completely clear, but it has been suggested that Cps14F ~~can enhance~~ has a role in glycosyltransferase activity. The *cps14G* gene of *S. pneumoniae* was shown to encode β -1,4-galactosyltransferase activity. In *S. pneumoniae* type ~~14~~ this 14, this activity is required for the second step in the biosynthesis of the oligosaccharide subunit (20). Based on the similarity ~~data~~

~~found~~ of the data, similar glycosyltransferase and enhancing activities are suggested for the ~~eps1G~~ *cps1G* and *cps1F* genes of *S. suis* type 1.

[00149] The protein encoded by the *cps1H* gene showed similarity to the Cps14H protein of *S. pneumoniae* (20). Based on ~~sequence-similarity~~ similarity, Cps14H was proposed to be the polysaccharide polymerase (20).

[00150] The protein encoded by the *cps1I* gene showed some similarity with the Cps14J protein of *S. pneumoniae* (19). The *cps14J* gene was shown to encode a β -1,4-galactosyltransferase activity, responsible for the addition of the fourth (~~i.e.~~ i.e. last) sugar in the synthesis of the *S. pneumoniae* serotype 14 polysaccharide.

[00151] Between Cps1G and ~~Cps1H~~ Cps1H, a gap of 718 bp was found. This region revealed three small Orfs. The three Orfs were expressed in three different reading frames and were not preceded by potential ribosome binding sites, nor contained potential start sites. However, the three potential gene products encoded by this region showed some similarity with three successive regions of the C-terminal part of the EpsK protein of *Streptococcus thermophilus* (27% identity, 40). The region related to the first 82 amino acids is lacking.

[00152] **Analysis of the cloned *cps9* genes.** We also determined the complete nucleotide sequence of the inserts of pCPS9-1 and pCPS9-2 (~~figure~~ (FIG. 6)). Examination of the sequence revealed the presence of three complete and two incomplete Orfs (~~Fig. 1C~~) (FIG. 1, part C). As in serotypes 1 and 2, all Orfs are preceded by a ribosome-binding site and are very closely coupled. As suggested by the hybridization data (~~Table 4~~) (Table 4), the Cps2D and Cps9D proteins were highly related (Table 2). Based on ~~sequence-comparisons~~ comparisons, pCPS9-1 lacked the first 27 amino acids of the Cps9D protein.

[00153] The protein encoded by the *cps9E* gene showed some similarity with the CapD protein of *Staphylococcus aureus* serotype 1 (24). Based on ~~sequence similarity-data~~ data, the Cap1D protein was suggested to be an epimerase or a dehydratase involved in the synthesis of N-acetylfructosamine or N- acetylgalactosamine (63).

[00154] Cps9F showed some similarity to the CapM proteins of *S. aureus* serotypes 5 and 8 (61, 64, 65). Based on ~~sequence similarity-data~~ data, Cap5M and Cap8M are proposed to be glycosyltransferases (63).

[00155] The protein encoded by the *cps9G* gene showed some similarity ~~with~~ to a protein of *Actinobacillus actinomycetemcomitans* (AB002668_4). This protein is part of a gene cluster responsible for the ~~serotype b-specific~~ serotype b-specific antigens of *Actinobacillus actinomycetemcomitans* ~~actinomycetemcomitans~~ actinomycetemcomitans. The function of the protein is unknown.

[00156] The protein encoded by the *cps9H* gene showed some similarity ~~with~~ to the *rfbB* gene of *Yersinia enterocolitica* (68). The RfbB protein was shown to be essential for O-antigen synthesis, but the function of the protein in the synthesis of the O:3 lipopolysaccharide is unknown.

[00157] **Serotype 1 and serotype 9-specific 9-specific *cps* genes.** To determine whether the cloned fragments in pCPS1-1, pCPS1-2, pCPS9-1 and pCPS9-2 contained the type-specific genes for serotype 1 and 9, respectively, ~~cross hybridization~~ cross-hybridization experiments were performed. DNA fragments of the individual *cps1* and *cps9* genes were amplified by PCR, ~~labelled~~ labeled with ^{32}P , and used to probe Southern blots of chromosomal DNA of the reference strains of the 35 different *S. suis* serotypes. The results are shown in Table 5. Based on the data obtained with the *cps2E* probe (Table 4), the *cps1E* probe was expected to hybridize with chromosomal DNA of *S. suis* serotypes 1, 2, 14, 27 and 1/2. The *cps1H*, *cps9E* and *cps9F* probes hybridized with most other serotypes. However, the *cps1F* and *cps1G* and *cps1I* probes hybridized with chromosomal DNA of serotypes 1 and 14 only. The *cps9G* and *cps9H* ~~probe~~ probes hybridized with serotype 9 only. These data suggest that the *cps9G* and *cps9H* probes are specific for serotype 9 ~~and therefore~~ and, therefore, could be useful tools for the development of rapid and sensitive diagnostic tests for *S. suis* type 9 infections.

[00158] **~~Type-specific~~ Type-specific PCR.** So far, the probes were tested on the 35 different reference strains only. To test the diagnostic value of the type-specific *cps* probes further, several other *S. suis* serotype 1, 2, 1/2, 9 and 14 strains were used. Moreover, since a ~~PCR-based~~ PCR-based method would be even more rapid and sensitive than a hybridization test, we tested whether we could use a PCR for the serotyping of the *S. suis* strains. The oligonucleotide primer sets were chosen within the *cps2J*, *cps1I* and *cps9H* genes. Amplified fragments of 675 bp, 380 bp and 390 bp were ~~expected~~ expected, respectively. The results show that 675 bp fragments were amplified on type 2 and 1/2 strains using *cps2J* primers; 380 bp fragments were amplified on type 1

and 14 strains using *cps11* primers and 390 bp fragments were amplified on type 9 strains using *cps9H* primers.

[00159] Construction of mutants impaired in capsule production. To evaluate the role of the capsule of *S. suis* type 2 in the ~~in~~ pathogenesis, we constructed two isogenic mutants in which capsule production was disturbed. To construct mutant 10cpsB, pCPS11 was used. In this ~~plasmid~~ plasmid, a part of the *cps2B* gene was replaced by the spectinomycin-resistance gene. To construct mutant strain ~~10cpsEF~~ 10cpsEF, the plasmid pCPS28 was used. In ~~pCPS28~~ pCPS28, the 3'-end of *cps2E* ~~gene~~ gene, as well as the 5'-end of *cps2F* ~~gene~~ gene, were replaced by the spectinomycin-resistance gene. pCPS11 and pCPS28 were used to electrotransform strain 10 of *S. suis* type 2 and spectinomycin-resistant colonies were selected. Southern blotting and hybridization experiments were used to select double ~~cross-over~~ cross-over integration events (results not shown). To test whether the capsular structure of the strains 10cpsB and 10cpsEF was disturbed, we used a slide agglutination test using a suspension of the mutant strains in hyperimmune ~~anti-*S. suis*~~ anti-*S. suis* type 2 serum (44). The results showed that even in the absence of ~~serotype-specific~~ serotype-specific antisera, the bacteria agglutinated. This indicates ~~that~~ that, in the mutant ~~strains~~ strains, the capsular structure was disturbed. To confirm this, thin sections of ~~wild-type~~ wild-type and mutant strains were compared by electron microscopy. The results showed ~~that~~ that, compared to the ~~wild-type~~ wild-type (Fig. 3A) wild-type (FIG. 3, part A), the amount of capsule produced by the mutant strains was greatly reduced (~~Figs. 3B and 3C~~) (FIG. 3, parts B and C). Almost no capsular material could be detected on the surface of the mutant strains.

Capsular mutants are sensitive to phagocytosis and killing by porcine alveolar macrophages (PAM). (“PAM”).

[00160] The capsular mutants were tested for their ability to resist phagocytosis by PAM in the presence of porcine SPF serum. The ~~wild-type~~ wild-type strain 10 seemed to be resistant to phagocytosis under these conditions (~~Fig. 4A~~) (FIGS. 9A and 9B). In contrast, the mutant strains were efficiently ingested by macrophages (~~Fig. 4A~~) (FIGS. 9A and 9B). After 90 ~~min~~ minutes, more than 99.7% (strain 10cpsB) and 99.8% (strain 10cpsEF) of the mutant cells were ingested by the macrophages. Moreover, as shown in ~~Fig. 4B~~ FIGS. 9A and 9B, the ingested strains were

efficiently killed by the macrophages. 90-98% of all ingested cells were killed within 90 min. No differences could be observed between ~~wild-type~~ wild-type and mutant strains. These data indicate that the capsule of *S. suis* type 2 efficiently protects the organism from uptake by macrophages *in vitro*.

[00161] Capsular mutants are less virulent for germfree piglets. The virulence properties of the wild-type and mutant strains were tested after experimental infection of newborn germfree pigs (45, 49). Table 1 shows that specific and nonspecific signs of disease could be observed in all pigs inoculated with the ~~wild-type~~ wild-type strain. Moreover, all pigs inoculated with the ~~wild-type~~ wild-type strain died during the course of the experiment or were killed because of serious illness or nervous disorders (Table 3). In contrast, the pigs inoculated with strains 10cpsB and 10cpsEF showed no specific signs of disease and all pigs survived until the end of the experiment (Table 6). The temperature of the pigs inoculated with the ~~wild-type~~ wild-type strain increased 2 days after inoculation and remained high until day 5 (Table 3). The temperature of the pigs inoculated with the mutant strains sometimes ~~exceeded the~~ exceeded 40°C, however, we could observe significant differences in the fever index (~~i.e. %~~ (i.e., percent of observations in an experimental group during which pigs showed fever (>40°C)) between pigs inoculated with ~~wild-type~~ wild-type and mutant strains. All pigs showed increased numbers of polymorphonuclear leucocytes (PMLs) (>10 x 10⁹ PMLs per ~~litre~~ liter) (Table 3). However, in pigs inoculated with the mutant ~~strains~~ strains, the percentage of samples with increased numbers of PMLs was considerably lower. *S. suis* strains and *B. bronchiseptica* could be isolated from the nasopharynx and feces swab samples of all pigs from 1 day post-infection until the end of the experiment (Table 3). Postmortem, the ~~wild-type~~ wild-type strain could frequently be isolated from the central nervous system (~~CNS~~), (“CNS”), kidney, heart, ~~liver~~, liver, spleen, serosae, joints and tonsils. Mutant strains could easily be recovered ~~from~~ from the tonsils, but were never recovered from the kidney, liver or spleen. Interestingly, low numbers of the mutant strains were isolated from the CNS, the serosae, the joints, the lungs and the heart. Taken together, these data strongly indicated that mutant *S. suis* strains, impaired in capsule production, are not virulent for young germfree pigs.

[00162] We describe the identification and the molecular ~~characterisation~~ characterization of the *cps* locus, involved in the capsular polysaccharide biosynthesis, of *S. ~~suis~~ suis*. Most of the

genes seemed to belong to a single transcriptional unit, suggesting a ~~co-ordinate~~ coordinate control of these genes. ~~We assign functions~~ Functions to most of the gene products were assigned. ~~We thereby identified regions~~ Regions involved in regulation (Cps2A), chain length determination (Cps2B, C), export (Cps2C) and biosynthesis (Cps2E, F, G, H, J, K) were identified. The region involved in biosynthesis is located at the ~~centre~~ center of the gene cluster and is flanked by two regions containing genes with more common functions. The incomplete *orf2Z* gene was located at the 5'-end of the cloned fragment. Orf2Z showed some similarity with the YitS protein of *B. subtilis*. However, because the function of the YitS protein is ~~unknown~~ unknown, this did not give us any information about the possible function of Orf2Z. Because the *orf2Z* gene is not a part of the *cps* operon, a role of this gene in polysaccharide biosynthesis is not expected. The Orf2Y protein showed some similarity with the YcxD protein of *B. subtilis* ~~(53)~~. ~~The~~ (53). The YcxD protein was suggested to be a regulatory protein. Similarly, Orf2Y may be involved in the regulation of polysaccharide biosynthesis. The Orf2X protein showed similarity with the YAAA proteins of *H. influenzae* and *E. coli*. The function of these proteins is unknown. In *S. suis* type ~~2~~ the 2, the *orf2X* gene seemed to be the first gene in the *cps2* operon. This suggests a role of Orf2X in the polysaccharide biosynthesis. In *H. influenzae* and *E. coli*, however, these proteins are not associated with capsular gene clusters. The analysis of isogenic mutants impaired in the expression of Orf2X should give more insight in the presumed role of Orf2X in the polysaccharide biosynthesis of *S. suis* type 2.

[00163] The gene products encoded by the *cps2E*, *cps2F*, *cps2G*, *cps2H*, *cps2J* and *cps2K* genes showed little similarity with glycosyltransferases of several Gram-positive or Gram-negative bacteria (18, 19, 20, 22, 25). The *cps2E* gene product shows some similarity with the Cps14E protein of *S. pneumoniae* (18, 19). Cps14E is a glucosyl-1-phosphate transferase that links glucose to a lipid carrier (18). In *S. pneumoniae* this is the first step in the biosynthesis of the oligosaccharide repeating unit. The structure of the *S. suis* serotype 2 capsule contains glucose, galactose, rhamnose, N-acetyl ~~glucoseamine~~ glucosamine and sialic acid in a ratio of 3:1:1:1:1 (7). Based on these ~~data~~ data, we conclude that Cps2E of *S. suis* has glucosyltransferase ~~activity~~, activity and is involved in the linkage of the first sugar to the lipid carrier.

[00164] The C-terminal region of the *cps2F* gene product showed some similarity with the RfbU of *Salmonella enteritica*. RfbU was shown to have mannosyltransferase activity (24). Because mannosyl is not a component of the *S. suis* type 2 ~~polysaccharide~~ polysaccharide, a mannosyltransferase activity is not expected in this organism. Nevertheless, *cps2F* encodes a glycosyltransferase with another sugar specificity.

[00165] Cps2G showed moderate similarity to a family of gene products suggested to encode galactosyltransferase activities (22, 24, 40). ~~Hence~~ Hence, a similar activity is shown for Cps2G.

[00166] Cps2H showed some similarity with LgtD of *H. influenzae* (U32768). Because LgtD was proposed to have glycosyltransferase ~~activity~~, activity, a similar activity is fulfilled by Cps2H.

[00167] Cps2J and Cps2K showed similarity to Cps14J of *S. pneumoniae* (20). Cps2J showed similarity with Cps14I of *S. pneumoniae* as well. Cps14I was shown to have N-acetyl glucosaminyltransferase activity, whereas Cps14J has a β -1,4-galactosyltransferase activity (20). In ~~*S. pneumoniae pneumoniae*~~, Cps14I is responsible for the addition of the third sugar and Cps14J for the addition of the last sugar in the synthesis of the type 14 repeating unit (20). Because the capsule of *S. suis* type 2 contains galactose as well as N-acetyl glucosamine components, galactosyltransferase as well as N-acetyl glucoaminyltransferase activities could be envisaged for the *cps2J* and *cps2K* gene products, respectively. As was observed for Cps14I and Cps14J, the N-termini of Cps2J and Cps2K showed a significant degree of sequence similarity. Within the N-terminal domains of Cps14I and Cps14J, two small regions were identified, which were also conserved in several other glycosyltransferases (22). Within these two regions, two Asp residues were proposed to be important for catalytic activity. The two conserved regions, DXS and DXDD, were also found in Cps2J and Cps2K.

[00168] The function of Cps2I remains unclear. Cps2I showed some similarity with a protein of *A. actinomycetemcomitans*. Although this protein part is of the gene cluster responsible for the serotype-B-specific antigens, the function of the protein is unknown.

[00169] We further describe the identification and characterization of the *cps* genes specific for *S. suis* serotypes 1, 2 and 9. After the entire *cps2* locus of *S. suis* serotype 2 was cloned and

characterized, functions for most of the *cps2* gene products could be assigned by sequence homologies. Based on these ~~data~~ data, the glycosyltransferase activities, required for type specificity, could be located in the ~~centre~~ center of the operon. Cross-hybridization experiments, using the individual *cps2* genes as probes on chromosomal DNAs of the 35 different serotypes, confirmed this idea. The regions containing the type-specific genes of serotypes 1 and 9 could be cloned and characterized, showing that an identical genetic organization of the *cps* operons of other *S. suis* serotypes exists. The *cps1E*, *cps1F*, *cps1G*, *cps1H*, and *cps1I* genes revealed a striking similarity with ~~*eps14E*~~ *cps14E*, *cps14F*, *cps14G*, *cps14H* and *cps14J* genes of *S. pneumoniae*. Interestingly, *S. pneumoniae* serotype 14 is the serotype most commonly associated with pneumococcal infections in young children (54), whereas *S. suis* serotype 1 strains are most commonly isolated from piglets younger than 8 weeks (46). In ~~*S. pneumoniae*~~ *S. pneumoniae*, the *cps14E*, *cps14G*, *cps14I* and *cps14J* encode the glycosyltransferases required for the synthesis of the type 14 tetrameric repeating unit, showing that the *cps1E*, *cps1G* and *cps1I* genes encoded glycosyltransferases. The precise functions of these genes as well as the substrate specificities of the enzymes can be established. In ~~*S. pneumoniae*~~ *S. pneumoniae*, the *cps14E* gene was shown to encode a glucosyl-1-phosphate transferase catalyzing the transfer of glucose to a lipid carrier. Moreover, *cpsE*-like genes were found in *S. pneumoniae* serotypes 9N, 13, 14, 15B, 15C, 18F, 18A and 19F (60). *CpsE* mutants were constructed in the serotypes 9N, ~~13~~, 13, 14 and 15B. All mutant strains lacked glycosyltransferase activity (60). Moreover, in all these ~~*S. pneumoniae* serotypes~~ serotypes, the *cpsE* gene seemed to be responsible for the addition of glucose to the lipid carrier. Based on these ~~data~~ data, we suggest that in *S. suis* ~~type 1~~ type 1, the *cps1E* gene may ~~fulfil~~ fulfill a similar function. The structure of the *S. suis* type 1 capsule is unknown, but it is composed of glucose, galactose, N-acetyl glucosamine, N-acetyl galactosamine and sialic acid in a ratio of 1: 2.4: 1: 1: 1.4 (5). ~~Therefore~~ Therefore, a role of a *cpsE*-like glycosyltransferase activity can easily be envisaged. ~~*CpsE* like~~ *CpsE*-like sequences were also found in serotypes 2, 1/2 and 14.

[00170] For polysaccharide biosynthesis in *S. pneumoniae* type 14, transfer of the second sugar of the repeating unit to the first lipid-linked sugar is performed by the gene products of *cps14F* and *cps14G* (20). Similar to Cps14F and Cps14G, the *S. suis* type 1 proteins Cps1F and Cps1G may act as one glycosyltransferase performing the same reaction. Cps14F and Cps14G of *S. pneumoniae*

showed similarity to the N-terminal half and C-terminal half of the SpsK protein of *Sphingomonas* (20, 67), respectively. This suggests a combined function for both proteins. Moreover, ~~*cps14F* and *cps14G* like~~ *cps14F*- and *cps14G*-like sequences were found in several serotypes of *S. pneumoniae* and these genes always seemed to exist together (60). The same was observed for *S. suis* type 1. The *cps1F* and *cps1G* probes hybridized with type 1 and type 14 strains.

[00171] According to the similarity found between the *cps1H* gene and the *cps14H* gene of *S. pneumoniae* (20), *cps1H* is expected to encode a polysaccharide polymerase.

[00172] The protein encoded by the *cps1I* gene showed some similarity with the Cps14J protein of *S. pneumoniae* (19). The *cps14J* gene was shown to encode a β -1,4-galactosyltransferase activity, responsible for the addition of the fourth-~~(i.e. (i.e. last)~~ sugar in the synthesis of the *S. pneumoniae* serotype 14 polysaccharide. In *S. suis* type-2~~the 2~~, the proteins encoded by the *cps2J* and *cps2K* genes showed similarity to the Cps14J protein. However, no significant homologies were found between Cps2J, Cps2K and Cps1I. In the N-terminal regions of Cps14J and ~~Cps14I~~ Cps14I, two small conserved regions, DXS and DXDD, were identified (19). These regions seemed to be important for catalytic activity (13). At the same positions in the ~~sequence~~ sequence, Cps2I contained the regions DXS and DXED.

[00173] In the region between Cps1G and ~~Cps1H~~ Cps1H, three small Orfs were identified. Since the Orfs were expressed in three different reading frames, and did not contain potential start sites, expression is not expected. However, the three potential gene products encoded by this region showed some similarity with three successive regions of the C-terminal part of the EpsK (protein of *Streptococcus thermophilus* (27% identity, 40). The region related to the first 82 amino acids is lacking. The EpsK protein was suggested to play a role in the export of the exopolysaccharide by rendering the polymerized exopolysaccharide more hydrophobic through a lipid modification. These data could suggest that the sequences in the region between Cps1G and Cps1H originated from an *epsK*-like sequence. Hybridization experiments showed that this *epsK*-like region is also present in other serotype 1 strains as well as in serotype 14 strains (results not shown).

[00174] The function of most of the cloned serotype 9 genes can be established. Based on sequence similarity ~~data~~ data, the *cps9E* and *cps9F* genes could be glycosyltransferases (61, 24, 63,

64, 65). Moreover, the *cps9G* and *cps9H* genes showed similarity to genes located in regions involved in polysaccharide biosynthesis, but the function of these genes is unknown (68).

[00175] Cross-hybridization experiments using the individual *cps2*, *cps1* and *cps9* genes as probes showed that the *cps9G* and *cps9H* probes specifically hybridized with serotype 9 strains. Therefore, these are useful as tools for the identification of *S. suis* type 9 strains both for diagnostic purposes as well as in epidemiological and transmission studies. We previously developed a PCR method which can be used to detect *S. suis* strains in nasal and tonsil swabs of pigs (62). The method was ~~for example~~ used to identify pathogenic (EF-positive) strains of *S. suis* serotype-2. ~~During the last years, beside 2.~~ Besides *S. suis* type 2 strains, serotype 9 strains are frequently isolated from organs of diseased pigs. However, ~~until now~~ now, a rapid and sensitive diagnostic test was not available for type 9 strains. Therefore, the ~~type-9 specific~~ 9-specific probes or the ~~type-9 specific~~ 9-specific PCR is of great diagnostic value. The *cps1F*, *cps1G* and *cps1I* probes hybridized with serotype 1 as well as with serotype 14 strains. In ~~coagglutination tests~~ tests, type 1 strains react with the anti-type 1 as well as with the anti-type 14 antisera (56). This suggests the presence of common epitopes between these serotypes. On the ~~other hand~~ hand, type 1 strains agglutinated only with anti-type 1 serum (56, 57), indicating that it is possible to detect differences between those serotypes.

[00176] The *cps2F*, *cps2G*, *cps2H*, *cps2I* and *cps2J* probes hybridized with serotypes 2 and 1/2 only. Serotype 34 showed a weak hybridizing signal with the *cps2G* probe. As shown in ~~agglutination tests~~ tests, type 1/2 strains react with sera directed against type 1 as well as with sera directed against type 2 strains (46). Therefore, type 1/2 shared antigens with both types 1 and 2. Based on the hybridization patterns of serotype 1/2 strains with the ~~*cps1*- and *cps2*-specific~~ *cps1*- and *cps2*-specific genes, serotype 1/2 seemed to be more closely related to type 2 strains than to type 1 strains. In our ~~current studies~~ studies, we identify type-specific genes, primers or probes which are used for the discrimination of serotypes 1, 14 and 2 and 1/2 and others of the 35 serotypes yet known. Furthermore, type-specific genes, primers or probes can now easily be developed for yet unknown serotypes, once they become isolated.

Cloning and characterization of a further part of the cps2 locus.

[00177] Based on the established ~~sequence~~ sequence, 11 genes, designated cps2L to cps2T, orf2U and ~~orf2V~~, orf2V, were identified. A gene homologous to genes involved in the polymerization of the repeating oligosaccharide unit (cps2O) as well as genes involved in the synthesis of sialic acid (cps2P to cps2T) were identified. Moreover, hybridization experiments showed that the genes involved in the sialic acid synthesis are present in ~~S. suis serotype~~ S. suis serotypes 1, 2, 14, 27 and 1/2. The “cps2M” and “cps2N” regions showed similarity to proteins involved in the polysaccharide biosynthesis of other ~~gram-positive~~ Gram-positive bacteria. However, these regions seemed to be truncated or were non-functional as the result of frame-shift or point mutations. At its ~~3'-end~~ 3'-end, the cps2 locus contained two insertional elements (“orf2U” and ~~“orf2V”~~) “orf2V”), both of which seemed to be non-functional.

[00178] To clone the remaining part of the cps2 locus, sequences of the 3'-end of pCPS26 (Fig. 1C) (FIG. 1, part C) were used to identify a chromosomal fragment containing cps2 sequences located further downstream. This fragment was cloned in ~~pKUN19~~ pKUN19, resulting in pCPS29. Using a similar ~~approach~~ approach, we subsequently isolated the plasmids pCPS30 and pCPS34 containing downstream cps2 sequences ~~(Fig. 1C)~~ (FIG. 1, part C).

Analysis of the cps2 operon.

[00179] The complete nucleotide sequence of the cloned fragments was determined. Examination of the compiled sequence revealed the presence ~~of~~ of a sequence encoding the C-terminal part of Cps2K, six apparently functional genes (designated cps2O-cps2T) and the remnants of 5 different ancestral genes (designated ~~“cps2L”, “cps2M”, “cps2N”, “cps2L”, “cps2M”, “cps2N”, “orf2U” and “orf2V”~~). The latter genes seemed to be truncated or incomplete as the result of the presence of stop codons or frame-shift mutations ~~(Fig. 1A)~~ (FIG. 1, part A). Neither potential promoter sequences nor potential stem-loop structures could be identified within the sequenced region. A ribosome-binding site precedes each ORF and the majority of the ORFs ~~is~~ are very closely linked. Three intergenic gaps were found: one between “cps2M” and “cps2N” (176 nucleotides), one between cps2O and cps2P (525 nucleotides), and one between cps2T and “orf2U”

(200 nucleotides). These and our above data show that Orf2X and Cps2A-Orf2T are part of a single operon.

[00180] A list of all loci and their properties is shown in Table 4. The “cps2L” region contained three potential ~~ORFs~~, ORFs of 103, 79 and 152 amino acids, respectively, which were only separated from each other by stop codons. Only the first ORF is preceded by a potential ribosomal binding site and contained a methionine start codon. This suggests that “cps2L” originates from an ancestral cps2L gene, which coded for a protein of 339 amino acids. The function of this hypothetical cps2L protein remains unclear so far: no significant homologies were found between Cps2L and proteins present in the data libraries. It is not clear whether the first ORF of the “cps2L” region is expressed into a protein of 103 amino acids. ~~The “cps2M”~~ The “cps2M” region showed homology to the N-terminal 134 amino acids of the NeuA proteins of ~~Streptococcus agalactiae and Escherichia coli~~ Streptococcus agalactiae and Escherichia coli (AB017355, 32). However, although the “cps2M” region contained a potential ribosome binding site, a methionine start codon was absent. Compared with the ~~S. agalactiae~~ S. agalactiae sequence, the ATG start codon was replaced by a lysin encoding AAG codon. Moreover, the region homologous to the first 58 amino acids of the ~~S. agalactiae~~ S. agalactiae NeuA (identity 77%) was separated from the region homologous to amino acids 59-134 of NeuA by a repeated DNA sequence of 100-bp (~~see later~~). (see herein). In addition, the region homologous to amino acids 59 to 95 of NeuA (identity 32%) and the region homologous to the amino acids 96 to 134 of NeuA (identity 50%) were present in different reading frames. Therefore, the partial and truncated NeuA homologue is probably nonfunctional in ~~S. suis~~. S. suis. The “cps2N” region showed homology to CpsJ of ~~S. agalactiae~~ S. agalactiae (accession no. AB017355). However, sequences homologous to the first 88 amino acids of CpsJ were lacking in ~~S. suis~~. S. suis. Moreover, the homologous region was present in two different reading frames. The protein encoded by the cps2O gene showed homology to proteins of several streptococci involved in the transport of the oligosaccharide repeating unit (accession no. AB017355), suggesting a similar function for Cps2O. The proteins encoded by the cps2P, cps2S and cps2T genes showed homology to the NeuB, NeuD and NeuA proteins of ~~S. agalactiae and E. coli~~ S. agalactiae and E. coli (accession no. AB017355). Because the “cps2M” region also showed homology to NeuA of ~~E. coli, the S. suis~~ E. coli, the S. suis cps2 locus contains a functional neuA

gene (cps2T) as well as a nonfunctional (“cps2M”) gene. The mutual homology between these two regions showed an identity of 77% at the amino acid level over amino acids 1-58 and 49% over the amino acids 59-134. Cps2Q and Cps2R showed homology to the N-terminal and C-terminal parts of the NeuC protein of ~~S. agalactiae and E. coli~~, S. agalactiae and E. coli, respectively. This suggests that the function of the ~~S. agalactiae~~ S. agalactiae NeuC protein in ~~S. suis~~ S. suis is likely fulfilled by two different proteins. In ~~E. coli~~ E. coli, the neu genes are known to be involved in the synthesis of sialic acid. NeuNAc is synthesized from N-acetylmannosamine and phosphoenolpyruvate by NeuNAc synthetase. Subsequently, NeuNAc is converted to CMP-NeuNAc by the enzyme CMP-NeuNAc synthetase. CMP-NeuNAc is the substrate for the synthesis of polysaccharide. In ~~E. coli~~ E. coli, K1 NeuB is the NeuNAc synthetase, and NeuA is the CMP-NeuNAc synthetase. NeuC has been implicated in the NeuNAc synthesis, but its precise role is not known. The precise role of NeuD is not known. A role of the Cps2P-Cps2T proteins in the synthesis of sialic acid can easily be envisaged, since the capsule of ~~S. suis~~ S. suis serotype 2 is rich in sialic acid. In ~~S. agalactiae~~ S. agalactiae, sialic acid has been shown to be critical to the virulence function of the type III capsule. Moreover, it has been suggested that the presence of sialic acid in the capsule of bacteria which can cause meningitis may be important for the capacity of these bacteria to breach the blood-brain barrier. So far, however, the requirement of the sialic acid for virulence of ~~S. suis~~ S. suis remains unclear.

[00181] “Orf2U” and “Orf2V” showed homology to proteins located on two different insertional elements. “Orf2U” is homologous to IS1194 of ~~Streptococcus thermophilus~~, Streptococcus thermophilus, whereas “Orf2V” showed homology to a putative transposase of ~~Streptococcus pneumoniae~~, Streptococcus pneumoniae. This putative transposase was recently found to be associated with the type 2 capsular locus of ~~S. pneumoniae~~, S. pneumoniae. Compared with the original insertional elements in ~~S. thermophilus and S. pneumoniae~~, S. thermophilus and S. pneumoniae, both “Orf2U” and “Orf2V” are likely to be non-functional due to frame shift mutations within their coding regions.

[00182] A striking observation was the presence of a sequence of 100 bp (~~Fig. 9~~) (FIG. 10) which was repeated three times within the cps2 operon. The sequence is highly conserved (between 94% and ~~98%~~) 98%) and was found in the intergenic regions between cps2G and cps2H, within

“cps2M” and between cps2O and cps2P. No significant homologies were found between this 100-bp direct repeat sequence and sequences present in the data libraries, suggesting that the sequence is unique for ~~S. suis~~ *S. suis*.

~~Distribution of the cps2 sequences among the 35 S. suis serotypes.~~ **Distribution of the cps2 sequences among the 35 S. suis serotypes.**

[00183] To examine the presence of sialic acid encoding genes in other ~~S. suis~~ *S. suis* serotypes, we performed cross-hybridization experiments. DNA fragments of the individual cps2 genes were amplified by PCR, ~~radiolabelled~~ radiolabeled with ³²P and hybridized to chromosomal DNA of the reference strains of the 35 different ~~S. suis~~ *S. suis* serotypes. As a ~~positive-control~~ control, we used a probe specific for ~~S. suis~~ *S. suis* 16S rRNA. The 16S rRNA probe hybridized with almost equal intensities to all serotypes tested (Table 4). The “cps2L” sequence hybridized with DNA of ~~serotype~~ serotypes 1, 2, 14 and 1/2. The “cps2M”, cps2O, cps2P, cps2Q, cps2R, cps2S and cps2T genes hybridized with DNA of ~~serotype~~ serotypes 1, 2, 14, 27 and 1/2. Because the cps2P-cps2T genes are most ~~probably~~ likely involved in the synthesis of sialic ~~acid~~ acid, these results suggest that sialic acid is also a part of the capsule in the ~~S. suis serotype~~ *S. suis* serotypes 1, 2, 14, 27 and 1/2. This is in agreement with the finding that the serotypes 1, 2 and 1/2 possess a capsule that is rich in sialic acid. Although the chemical compositions of the capsules of ~~serotype~~ serotypes 14 and 27 are unknown, recent agglutination studies using sialic acid-binding lectins suggested the presence of sialic acid in ~~S. suis~~ *S. suis* serotype 14, but not in serotype 27. In these studies, sialic acid was also detected in serotypes 15 and 16. Since the latter observation is not in agreement with our hybridization studies, it might be that other genes, not homologous to the cps2P-cps2T genes, are responsible for the sialic acid synthesis in serotypes 15 and 16.

[00184] A probe based ~~on “cps2N”~~ on “cps2N” sequences hybridized with DNA from serotypes 1, 2, 14 and 1/2. A probe specific for “orf2U” hybridized with serotypes 1, 2, 7, 14, 24, 27, 32, 34, and 1/2, whereas a probe specific for “orf2V” hybridized with many different serotypes. In addition, we prepared a probe specific for the 100-bp direct repeat sequence. This probe hybridized with the serotypes 1, 2, 13, 14, 22, 24, 27, 29, 32, 34 and 1/2 (Table 4). To analyze the number of copies of the direct repeat sequence within the ~~S. suis~~ *S. suis* serotype 2 chromosome, a Southern

blot hybridization and analysis was performed. Therefore, chromosomal DNA of ~~*S. suis*~~ *S. suis* serotype 2 was digested with ~~*NotI*~~ *NcoI* and hybridized with a ~~32P-labelled~~ 32P-labeled direct repeat sequence. Only one hybridizing fragment, containing the three direct repeats present on the *cps2* locus, was found (results not shown). This indicates that the 100-bp direct repeat sequence is only associated with the *cps2* locus. In ~~*S. pneumoniae*~~ *S. pneumoniae*, a 115-bp long repeated sequence was found to be associated with the capsular genes of serotypes 1, 3, 14 and 19F. In ~~*S. pneumoniae*~~ *S. pneumoniae*, this 115-bp sequence was also found in the vicinity of other genes involved in pneumococcal virulence (hyaluronidase and neuraminidase genes). A regulatory role of the 115-bp sequence in ~~co-ordinate~~ coordinate control of these virulence-related genes was suggested.

[00185] To study the role of the capsule in resistance to phagocytosis and in virulence, we constructed two isogenic mutants in which capsule synthesis was disturbed. In 10cpsB, the *cps2B* gene was disturbed by the insertion of an antibiotic-resistance gene, whereas in ~~10cpsEF~~ 10cpsEF, parts of the *cps2E* and *cps2F* genes were replaced. Both mutant strains seemed to be completely unencapsulated. Because the ~~*eps-2*~~ *cps2* genes seemed to be part of an ~~operon~~ operon, polar effects cannot be excluded. ~~Therefore~~ Therefore, these data did not give any information about the role of Cps2B, Cps2E or Cps2F in the polysaccharide biosynthesis. However, the results clearly show that the capsular polysaccharide of *S. suis* type 2 is a surface component with antiphagocytic activity. *In vitro* ~~wild-type~~ wild-type encapsulated bacteria are ingested by phagocytes at a very low frequency, whereas the mutant unencapsulated bacteria are efficiently ingested by porcine macrophages. Within 2 hours, over 99.6% of mutant bacteria were ingested and over 92% of the ingested bacteria were killed. Intracellularly, ~~wild-type~~ wild-type as well as mutant strains seemed to be killed with the same efficiency. This suggests that the loss of capsular material is associated with loss of capacity to resist uptake by macrophages. This loss of resistance to *in vitro* phagocytosis was associated with a substantial attenuation of the virulence in germfree pigs. All pigs inoculated with the mutant strains survived the experiment and did not show any specific clinical signs of disease. Only some aspecific clinical signs of disease could be observed. Moreover, mutant bacteria could be reisolated from the pigs. This supports the idea that, as in other pathogenic Streptococci, the capsule of *S. suis* acts as an important virulence factor. Transposon mutants prepared by Charland impaired in the capsule

production showed a reduced virulence in pigs and mice. To construct these ~~mutants~~ mutants, the type 2 reference strain S735 was used. We previously showed that this strain is only weakly virulent for young pigs. Moreover, the insertion site of the transposon is unsolved ~~so far~~, so far.

~~As a further example herein a rapid PCT test for *Streptococcus suis* type 7 is described.~~ **As a further example herein a rapid PCT test for *Streptococcus suis* type 7 is described.**

[00186] Recent epidemiological studies on ~~Streptococcus suis~~ *Streptococcus suis* infections in pigs indicated that, besides serotypes 1, 2 and 9, serotype 7 is also frequently associated with diseased animals. For the latter serotype, however, no rapid and sensitive diagnostic methods are available. This hampers prevention and control programs. Here we describe the development of a type-specific PCR test for the rapid and sensitive detection of ~~S. suis~~ *S. suis* serotype 7. The test is based on DNA sequences of capsular (cps) genes specific for serotype 7. These sequences could be identified by cross-hybridization of several individual cps genes with the chromosomal DNAs of 35 different ~~S. suis~~ *S. suis* serotypes.

[00187] ~~Streptococcus suis~~ *Streptococcus suis* is an important cause of meningitis, septicemia, arthritis and sudden death in young pigs [69, 70]. It can, however, also cause meningitis in man (71). Attempts to control the disease are still hampered by the lack of sufficient knowledge about the epidemiology of the disease and the lack of effective vaccines and sensitive diagnostics.

[00188] ~~S. suis~~ *S. suis* strains can be identified and classified by their morphological, biochemical and serological characteristics (70, 73, 74). Serological classification is based on the presence of specific antigenic determinants. Isolated and biochemically characterized ~~S. suis~~ *S. suis* cells are agglutinated with a panel of specific sera. These typing methods are very laborious and time-consuming and can only be performed on isolated colonies. Moreover, it has been reported that nonspecific cross-reactions may occur among different types of ~~S. suis~~ *S. suis* (75, 76).

[00189] So far, 35 different serotypes have been described (7, 78, 79). ~~S. suis~~ *S. suis* serotype 2 is the most prevalent type isolated from diseased pigs, followed by serotypes ~~9, and 9~~ 9 and 1. However, ~~recently~~ recently, serotype 7 strains were also frequently isolated from diseased pigs (80, 81, 82). This suggests that infections with ~~S. suis~~ *S. suis* serotype 7 strains seemed to be an

increasing problem. Moreover, the virulence of ~~S. suis~~ *S. suis* serotype 7 strains was confirmed by experimental infection of young pigs (83).

[00190] Recently, rapid and sensitive PCR assays specific for serotypes 2 (and 1/2), 1 (and 14) and 9 were developed (84). These assays were based on the cps loci of ~~S. suis~~ *S. suis* serotypes 2, 1 and 9 (84, 85). However, ~~until now~~ now, no rapid and sensitive diagnostic ~~test is~~ test was available for ~~S. suis~~ *S. suis* serotype 7. Herein we describe the development of a PCR test for the rapid and sensitive detection of ~~S. suis~~ *S. suis* serotype 7 strains. The test is based on DNA sequences which form a part of the cps locus of ~~S. suis~~ *S. suis* serotype 7. Compared with the serological serotyping ~~methods~~ methods, the PCR assay was a rapid, reliable and sensitive assay. Therefore, this test, in combination with the PCR tests which we previously developed for ~~serotype~~ serotypes 1, 2 and 9, will undoubtedly contribute to a more rapid and reliable diagnosis of ~~S. suis~~ *S. suis* and may facilitate control and eradication programs.

~~Materials and Methods~~ **Materials and Methods**

~~Bacterial strains, growth conditions and serotyping~~ **Bacterial strains, growth conditions and serotyping**

[00191] The bacterial strains and plasmids used in this study are listed in Table 7. The ~~S. suis~~ *S. suis* reference strains were obtained from M. Gottschalk, Canada. ~~S. suis~~ *S. suis* strains were grown in Todd-Hewitt broth (code CM189, Oxoid), and plated on Columbia agar blood base (code CM331, Oxoid) containing 6% (v/v) horse blood. ~~E. coli~~ *E. coli* strains were grown in Luria broth (86) and plated on Luria broth containing 1.5% (w/v) agar. If required, ampicillin was added to the plates. The ~~S. suis~~ *S. suis* strains were serotyped by the slide agglutination test with serotype-specific antibodies (70).

~~DNA techniques~~ **DNA techniques**

[00192] Routine DNA manipulations and PCR reactions were performed as described by Sambrook et al. (88). Blotting and hybridization ~~was~~ were performed as described previously (84, 86).

~~DNA sequence analysis.~~ DNA sequence analysis.

[00193] DNA sequences were determined on a 373A DNA Sequencing system (Applied Biosystems, Warrington, GB). Samples were prepared by use of ~~a~~ an ABI/PRISM dye terminator cycle sequencing ready reaction kit (Applied Biosystems). Custom-made sequencing primers were purchased from Life Technologies. Sequencing data were assembled and analyzed using the McMollyTetra program. The BLAST program was used to search for protein sequences homologous to the deduced amino acid sequences.

~~PCR.~~ PCR.

[00194] The primers used for the cps7H PCR correspond to the positions 3334-3354 and 3585-3565 in the ~~S. suis~~ S. suis cps7 locus. The sequences were: 5'-AGCTCTAACACGAAATAAGGC-3' (SEQ ID NO:7) and 5'-GTCAAACACCCTGGATAGCCG-3' (SEQ ID NO:8).

[00195] The reaction mixtures contained 10 mM Tris-HCl, pH 8.3; 1.5 mM MgCl₂; 50 mM KCl; 0.2 mM of each of the four deoxynucleotide triphosphates; 1 microM of each of the primers and 1U of AmpliTaq Gold DNA polymerase (Perkin Elmer Applied Biosystems, New Jersey). DNA amplification was carried out in a Perkin Elmer 9600 thermal cycler and the program consisted of an incubation for 10 min at ~~95°C~~ 95°C and 30 cycles of 1 min at ~~95°C~~ 95°C, 2 min at ~~56°C~~ 56°C and 2 min at ~~72°C~~ 72°C.

~~Results and discussion~~ Results and discussion

~~Cloning of the serotype 7-specific cps genes.~~ Cloning of the serotype 7-specific cps genes.

[00196] To isolate the type-specific cps genes of ~~S. suis~~ S. suis serotype ~~7~~ 7, we used the cps9E gene of serotype 9 as a probe to identify chromosomal DNA fragments of type 7 containing homologous DNA sequences (84). A 1.6-kb PstI fragment was identified and cloned in pKUN19. This yielded pCPS7-1 (~~Fig. 11C~~). (FIG. 11, part C). In turn, this fragment was used as a probe to identify an overlapping 2.7 kb ScaI-ClaI fragment. pGEM7 containing the latter fragment was designated pCPS7-2 (~~Fig. 11C~~). (FIG. 11, part C).

~~Analysis of the cloned cps7 genes.~~**Analysis of the cloned cps 7 genes.**

[00197] The complete nucleotide sequences of the inserts of pCPS7-1, pCPS7-2 were determined. Examination of the cps7 sequence revealed the presence of two complete and two incomplete open reading frames (ORFs) (~~Fig. 11C~~) (FIG. 11, part C). All ORFs are preceded by a ribosome-binding site. In accord with the data obtained for the cps1, cps2 and cps9 genes of serotypes 1, 2 and 9, respectively, the type 7 ORFs are very closely linked to each other. The only significant intergenic gap was that found between cps7E and cps7F (443 nucleotides). No obvious promoter sequences or potential stem-loop structures were found in this region. This suggests that, as in ~~serotype~~ serotypes 1, 2 and 9, the cps genes in serotype 7 form part of an operon.

[00198] An overview of the ORFs and their properties is shown in Table 8. As expected on the basis of the hybridization data (84), the Cps9E and Cps7E proteins showed a high similarity (identity 99%, Table 8). Based on sequence comparisons between Cps9E and Cps7E, the PstI fragment of pCPS7-1 lacks the region encoding the first 371 codons of Cps7E. The C-terminal part of the protein encoded by the cps7F gene showed some similarity with the BplG protein of ~~Bordetella pertussis~~ Bordetella pertussis (88), as well as with the C-terminal part of ~~S. suis~~ S. suis Cps2E (85). Both BplG and Cps2E were suggested to have glycosyltransferase activity and are probably involved in the linkage of the first sugar to the lipid carrier (85, 88). The protein encoded by the cps7G gene showed similarity with the BlpF protein of ~~Bordetella pertussis~~ Bordetella pertussis (88). ~~BplF~~ BlpF is likely to be involved in the biosynthesis of an amino sugar, suggesting a similar function for Cps7G. The protein encoded by the cps7H gene showed similarity with the WbdN protein of ~~E. coli~~ E. coli (89) as well as with the N-terminal part of the Cps2K protein of ~~S. suis~~ S. suis (81). Both WbdN and Cps2K were suggested to have glycosyltransferase activity (85, 89).

~~Serotype 7-specific cps genes.~~**Serotype 7-specific cps genes.**

[00199] To determine whether the cloned fragments in pCPS7-1 and pCPS7-2 contained serotype 7-specific DNA sequences, ~~cross-hybridization~~ cross-hybridization experiments were performed. DNA fragments of the individual cps7 genes were amplified by PCR, ~~labelled~~ labeled with 32P, and used to probe spot blots of chromosomal DNA of the reference strains of 35 different

~~S. suis~~ *S. suis* serotypes. The results are summarized in Table 9. As expected, based on the data obtained with the cps9E probe (84), the cps7E probe hybridized with chromosomal DNA of many different ~~S. suis~~ *S. suis* serotypes. The cps7F and cps7G probes showed hybridization with chromosomal DNA of ~~S. suis~~ *S. suis* serotypes 4, 5, 7, 17, and 23. However, the cps7H probe hybridized with chromosomal DNA of serotype 7 only, indicating that this gene is specific for serotype 7.

~~Type-specific PCR~~ **Type-specific PCR.**

[00200] We tested whether we could use PCR instead of hybridization for the typing of the ~~S. suis~~ *S. suis* serotype 7 strains. For that ~~purpose~~ purpose, we selected an oligonucleotide primer set within the cps7H gene with which an amplified fragment of 251-bp was expected. In addition, we included in our analysis several ~~S. suis~~ *S. suis* serotype 7 strains, other than the reference strain. These strains were obtained from different countries and were isolated from different organs (Table 7). The results show that indeed a fragment of about 250-bp was amplified with all type 7 strains used (~~Fig. 12B~~), (FIG. 12, part B), whereas no PCR products were obtained with serotype 1, 2 and 9 strains (~~Fig. 12A~~), (FIG. 12, part A). This suggests that the PCR test, as described here, is a rapid diagnostic tool for the identification of ~~S. suis~~ *S. suis* serotype 7 strains. ~~Until now~~ now, such a diagnostic test was not available for serotype 7 strains. Together with the recently developed PCR assays for ~~serotype~~ serotypes 1, 2, 1/2, 14 and 9, this assay may be an important diagnostic tool to detect pigs carrying serotype 2, 1/2, 1, ~~14~~, 14, 9 and 7 strains and may facilitate control and eradication programs.

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Thr Ser Leu His Gly Asn Tyr His Phe Pro Val Gly Gln Val His Leu
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 Asn Ser Asp Gln Ala Leu Gly Phe Val Arg Glu Arg Tyr Ser Leu Thr
 355 360 365
 Gly Gly Asp Asn Asp Arg Gly Lys Asn Gln Glu Lys Val Ile Ala Ala
 370 375 380
 Leu Ile Lys Lys Met Ser Thr Pro Glu Asn Leu Lys Asn Tyr Gln Ala
 385 390 395 400
 Ile Leu Ser Gly Leu Glu Gly Ser Ile Gln Thr Asp Leu Ser Leu Glu
 405 410 415
 Thr Ile Met Ser Leu Val Asn Thr Gln Leu Glu Ser Gly Thr Gln Phe
 420 425 430
 Thr Val Glu Ser Gln Ala Leu Thr Gly Thr Gly Arg Ser Asp Leu Ser
 435 440 445
 Ser Tyr Ala Met Pro Gly Ser Gln Leu Tyr Met Met Glu Ile Asn Gln
 450 455 460
 Asp Ser Leu Glu Gln Ser Lys Ala Ala Ile Gln Ser Val Leu Val Glu
 465 470 475 480
 Lys

<210> 13

<211> 229

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2B

<400> 13

Met Asn Asn Gln Glu Val Asn Ala Ile Glu Ile Asp Val Leu Phe Leu
 1 5 10 15
 Leu Lys Thr Ile Trp Arg Lys Lys Phe Leu Ile Leu Leu Thr Ala Val
 20 25 30

Leu	Thr	Ala	Gly	Leu	Ala	Phe	Val	Tyr	Ser	Ser	Phe	Leu	Val	Thr	Pro
		35					40					45			
Gln	Tyr	Asp	Ser	Thr	Thr	Arg	Ile	Tyr	Val	Val	Ser	Gln	Asn	Val	Glu
	50					55					60				
Ala	Gly	Ala	Gly	Leu	Thr	Asn	Gln	Glu	Leu	Gln	Ala	Gly	Thr	Tyr	Leu
65					70					75					80
Ala	Lys	Asp	Tyr	Arg	Glu	Ile	Ile	Leu	Ser	Gln	Asp	Val	Leu	Thr	Gln
				85					90					95	
Val	Ala	Thr	Glu	Leu	Asn	Leu	Lys	Glu	Ser	Leu	Lys	Glu	Lys	Ile	Ser
			100					105					110		
Val	Ser	Ile	Pro	Val	Asp	Thr	Arg	Ile	Val	Ser	Ile	Ser	Val	Arg	Asp
		115					120					125			
Ala	Asp	Pro	Asn	Glu	Ala	Ala	Arg	Ile	Ala	Asn	Ser	Leu	Arg	Thr	Phe
	130					135					140				
Ala	Val	Gln	Lys	Val	Val	Glu	Val	Thr	Lys	Val	Ser	Asp	Val	Thr	Thr
145					150					155					160
Leu	Glu	Glu	Ala	Val	Pro	Ala	Glu	Glu	Pro	Thr	Thr	Pro	Asn	Thr	Lys
				165					170					175	
Arg	Asn	Ile	Leu	Leu	Gly	Leu	Leu	Ala	Gly	Gly	Ile	Leu	Ala	Thr	Gly
			180					185					190		
Leu	Val	Leu	Val	Met	Glu	Val	Leu	Asp	Asp	Arg	Val	Lys	Arg	Pro	Gln
		195					200					205			
Asp	Ile	Glu	Glu	Val	Met	Gly	Leu	Thr	Leu	Leu	Gly	Ile	Val	Pro	Asp
	210					215					220				
Ser	Lys	Lys	Leu	Lys											
225															

<210> 14

<211> 225

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2C

<400> 14

Met	Ala	Met	Leu	Glu	Ile	Ala	Arg	Thr	Lys	Arg	Glu	Gly	Val	Asn	Lys	
1				5					10					15		
Thr	Glu	Glu	Tyr	Phe	Asn	Ala	Ile	Arg	Thr	Asn	Ile	Gln	Leu	Ser	Gly	
			20					25					30			
Ala	Asp	Ile	Lys	Val	Val	Gly	Ile	Thr	Ser	Val	Lys	Ser	Asn	Glu	Gly	
		35					40					45				
Lys	Ser	Thr	Thr	Ala	Ala	Ser	Leu	Ala	Ile	Ala	Tyr	Ala	Arg	Ser	Gly	
	50					55					60					
Tyr	Lys	Thr	Val	Leu	Val	Asp	Ala	Asp	Ile	Arg	Asn	Ser	Val	Met	Pro	
65					70					75					80	
Gly	Phe	Phe	Lys	Pro	Ile	Thr	Lys	Ile	Thr	Gly	Leu	Thr	Asp	Tyr	Leu	
				85					90					95		
Ala	Gly	Thr	Thr	Asp	Leu	Ser	Gln	Gly	Leu	Cys	Asp	Thr	Asp	Ile	Pro	
			100					105					110			
Asn	Leu	Thr	Val	Ile	Glu	Ser	Gly	Lys	Val	Ser	Pro	Asn	Pro	Thr	Ala	
		115					120					125				
Leu	Leu	Gln	Ser	Lys	Asn	Phe	Glu	Asn	Leu	Leu	Ala	Thr	Leu	Arg	Arg	
	130					135					140					
Tyr	Tyr	Asp	Tyr	Val	Ile	Val	Asp	Cys	Pro	Pro	Leu	Gly	Leu	Val	Ile	
145					150					155					160	
Asp	Ala	Ala	Ile	Ile	Ala	Gln	Lys	Cys	Asp	Ala	Met	Val	Ala	Val	Val	
				165					170					175		
Glu	Ala	Gly	Asn	Val	Lys	Cys	Ser	Ser	Leu	Lys	Lys	Val	Lys	Glu	Gln	
			180					185					190			
Leu	Glu	Gln	Thr	Gly	Thr	Pro	Phe	Leu	Gly	Val	Ile	Leu	Asn	Lys	Tyr	
		195					200					205				
Asp	Ile	Ala	Thr	Glu	Lys	Tyr	Ser	Glu	Tyr	Gly	Asn	Tyr	Gly	Lys	Lys	
	210					215					220					
Ala																
225																

Val	His	Cys	Val	Ala	Ser	Asp	Met	His	Asn	Leu	Tyr	Ser	Arg	Pro	Pro
		195					200					205			
Phe	Met	Arg	Glu	Ala	Tyr	Gln	Leu	Val	Lys	Lys	Glu	Tyr	Gly	Glu	Asp
	210					215					220				
Arg	Ala	Lys	Ala	Leu	Phe	Lys	Lys	Asn	Pro	Leu	Leu	Ile	Leu	Lys	Asn
225					230					235					240
Gln	Val	Gln													

<210> 16

<211> 459

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2E

<400> 16

Met	Asn	Ile	Glu	Ile	Gly	Tyr	Arg	Gln	Thr	Lys	Leu	Ala	Leu	Phe	Asp
1				5					10					15	
Met	Ile	Ala	Val	Thr	Ile	Ser	Ala	Ile	Leu	Thr	Ser	His	Ile	Pro	Asn
			20					25					30		
Ala	Asp	Leu	Asn	Arg	Ser	Gly	Ile	Phe	Ile	Ile	Met	Met	Val	His	Tyr
		35					40					45			
Phe	Ala	Phe	Phe	Ile	Ser	Arg	Met	Pro	Val	Glu	Phe	Glu	Tyr	Arg	Gly
	50					55					60				
Asn	Leu	Ile	Glu	Phe	Glu	Lys	Thr	Phe	Asn	Tyr	Ser	Ile	Ile	Phe	Val
65					70					75					80
Ile	Phe	Leu	Met	Ala	Val	Ser	Phe	Met	Leu	Glu	Asn	Asn	Phe	Ala	Leu
				85					90					95	
Ser	Arg	Arg	Gly	Ala	Val	Tyr	Phe	Thr	Leu	Ile	Asn	Phe	Val	Leu	Val
			100					105					110		
Tyr	Leu	Phe	Asn	Val	Ile	Ile	Lys	Gln	Phe	Lys	Asp	Ser	Phe	Leu	Phe
		115					120					125			

Ser	Thr	Thr	Tyr	Gln	Lys	Lys	Thr	Ile	Leu	Ile	Thr	Thr	Ala	Glu	Leu			
	130					135					140							
Trp	Glu	Asn	Met	Gln	Val	Leu	Phe	Glu	Ser	Asp	Ile	Leu	Phe	Gln	Lys			
145					150					155					160			
Asn	Leu	Val	Ala	Leu	Val	Ile	Leu	Gly	Thr	Glu	Ile	Asp	Lys	Ile	Asn			
				165					170					175				
Leu	Pro	Leu	Pro	Leu	Tyr	Tyr	Ser	Val	Glu	Glu	Ala	Ile	Gly	Phe	Ser			
			180					185					190					
Thr	Arg	Glu	Val	Val	Asp	Tyr	Val	Phe	Ile	Asn	Leu	Pro	Ser	Glu	Tyr			
		195					200					205						
Phe	Asp	Leu	Lys	Gln	Leu	Val	Ser	Asp	Phe	Glu	Leu	Leu	Gly	Ile	Asp			
	210					215					220							
Val	Gly	Val	Asp	Ile	Asn	Ser	Phe	Gly	Phe	Thr	Val	Leu	Lys	Asn	Lys			
225					230					235					240			
Lys	Ile	Gln	Met	Leu	Gly	Asp	His	Ser	Ile	Val	Thr	Phe	Ser	Thr	Asn			
				245					250					255				
Phe	Tyr	Lys	Pro	Ser	His	Ile	Trp	Met	Lys	Arg	Leu	Leu	Asp	Ile	Leu			
			260					265					270					
Gly	Ala	Val	Val	Gly	Leu	Ile	Ile	Ser	Gly	Ile	Val	Ser	Ile	Leu	Leu			
		275					280					285						
Ile	Pro	Ile	Ile	Arg	Arg	Asp	Gly	Gly	Pro	Ala	Ile	Phe	Ala	Gln	Lys			
	290					295					300							
Arg	Val	Gly	Gln	Asn	Gly	Arg	Ile	Phe	Thr	Phe	Tyr	Lys	Phe	Arg	Ser			
305					310					315					320			
Met	Phe	Val	Asp	Ala	Glu	Val	Arg	Lys	Lys	Glu	Leu	Met	Ala	Gln	Asn			
				325					330					335				
Gln	Met	Gln	Gly	Gly	Met	Phe	Lys	Met	Asp	Asn	Asp	Pro	Arg	Ile	Thr			
			340					345					350					
Pro	Ile	Gly	His	Phe	Ile	Arg	Lys	Thr	Ser	Leu	Asp	Glu	Leu	Pro	Gln			
		355					360					365						
Phe	Tyr	Asn	Val	Leu	Ile	Gly	Asp	Met	Ser	Leu	Val	Gly	Thr	Arg	Pro			
	370					375					380							
Pro	Thr	Val	Asp	Glu	Phe	Glu	Lys	Tyr	Thr	Pro	Ser	Gln	Lys	Arg	Arg			
385					390					395					400			

Leu Ser Phe Lys Pro Gly Ile Thr Gly Leu Trp Gln Val Ser Gly Arg
 405 410 415
 Ser Asp Ile Thr Asp Phe Asn Glu Val Val Arg Leu Asp Leu Thr Tyr
 420 425 430
 Ile Asp Asn Trp Thr Ile Trp Ser Asp Ile Lys Ile Leu Leu Lys Thr
 435 440 445
 Val Lys Val Val Leu Leu Arg Glu Gly Gly Gln
 450 455

<210> 17

<211> 389

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2F

<400> 17

Met Arg Thr Val Tyr Ile Ile Gly Ser Lys Gly Ile Pro Ala Lys Tyr
 1 5 10 15
 Gly Gly Phe Glu Thr Phe Val Glu Lys Leu Thr Glu Tyr Gln Lys Asp
 20 25 30
 Lys Ser Ile Asn Tyr Phe Val Ala Cys Thr Arg Glu Asn Ser Ala Lys
 35 40 45
 Ser Asp Ile Thr Gly Glu Val Phe Glu His Asn Gly Ala Thr Cys Phe
 50 55 60
 Asn Ile Asp Val Pro Asn Ile Gly Ser Ala Lys Ala Ile Leu Tyr Asp
 65 70 75 80
 Ile Met Ala Leu Lys Lys Ser Ile Glu Ile Ala Lys Asp Arg Asn Asp
 85 90 95
 Thr Ser Pro Ile Phe Tyr Ile Leu Ala Cys Arg Ile Gly Pro Phe Ile
 100 105 110
 Tyr Leu Phe Lys Lys Gln Ile Glu Ser Ile Gly Gly Gln Leu Phe Val
 115 120 125

<210> 18

<211> 385

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2G

<400> 18

Met Lys Lys Ile Leu Tyr Leu His Ala Gly Ala Glu Leu Tyr Gly Ala
1 5 10 15

Asp Lys Val Leu Leu Glu Leu Ile Lys Gly Leu Asp Lys Asn Glu Phe
20 25 30

Glu Ala His Val Ile Leu Pro Asn Asp Gly Val Leu Val Pro Ala Leu
35 40 45

Arg Glu Val Gly Ala Gln Val Glu Val Ile Asn Tyr Pro Ile Leu Arg
50 55 60

Arg Lys Tyr Phe Asn Pro Lys Gly Ile Phe Asp Tyr Phe Ile Ser Tyr
65 70 75 80

His His Tyr Ser Lys Gln Ile Ala Gln Tyr Ala Ile Glu Asn Lys Val
85 90 95

Asp Ile Ile His Asn Asn Thr Thr Ala Val Leu Glu Gly Ile Tyr Leu
100 105 110

Lys Arg Lys Leu Lys Leu Pro Leu Leu Trp His Val His Glu Ile Ile
115 120 125

Val Lys Pro Lys Phe Ile Ser Asp Ser Ile Asn Phe Leu Met Gly Arg
130 135 140

Phe Ala Asp Lys Ile Val Thr Val Ser Gln Ala Val Ala Asn His Ile
145 150 155 160

Lys Gln Ser Pro His Ile Lys Asp Asp Gln Ile Ser Val Ile Tyr Asn
165 170 175

Gly Val Asp Asn Lys Val Phe Tyr Gln Ser Asp Ala Arg Ser Val Arg
180 185 190

Glu	Arg	Phe	Asp	Ile	Asp	Glu	Glu	Ala	Leu	Val	Ile	Gly	Met	Val	Gly			
		195					200					205						
Arg	Val	Asn	Ala	Trp	Lys	Gly	Gln	Gly	Asp	Phe	Leu	Glu	Ala	Val	Ala			
	210					215					220							
Pro	Ile	Leu	Glu	Gln	Asn	Pro	Lys	Ala	Ile	Ala	Phe	Ile	Ala	Gly	Ser			
225					230					235					240			
Ala	Phe	Glu	Gly	Glu	Glu	Trp	Arg	Val	Val	Glu	Leu	Glu	Lys	Lys	Ile			
				245					250					255				
Ser	Gln	Leu	Lys	Val	Ser	Ser	Gln	Val	Arg	Arg	Met	Asp	Tyr	Tyr	Ala			
			260					265					270					
Asn	Thr	Thr	Glu	Leu	Tyr	Asn	Met	Phe	Asp	Ile	Phe	Val	Leu	Pro	Ser			
		275					280					285						
Thr	Asn	Pro	Asp	Pro	Leu	Pro	Thr	Val	Val	Leu	Lys	Ala	Met	Ala	Cys			
	290					295					300							
Gly	Lys	Pro	Val	Val	Gly	Tyr	Arg	His	Gly	Gly	Val	Cys	Glu	Met	Val			
305					310					315					320			
Lys	Glu	Gly	Val	Asn	Gly	Phe	Leu	Val	Thr	Pro	Asn	Ser	Pro	Leu	Asn			
				325					330					335				
Leu	Ser	Lys	Val	Ile	Leu	Gln	Leu	Ser	Glu	Asn	Ile	Asn	Leu	Arg	Lys			
			340					345					350					
Lys	Ile	Gly	Asn	Asn	Ser	Ile	Glu	Arg	Gln	Lys	Glu	His	Phe	Ser	Leu			
		355					360					365						
Lys	Ser	Tyr	Val	Lys	Asn	Phe	Ser	Lys	Val	Tyr	Thr	Ser	Leu	Lys	Val			
	370					375					380							
Tyr																		
385																		

<210> 19

<211> 456

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> cps2h

<400> 19

Met	Lys	Ile	Ile	Ser	Phe	Thr	Met	Val	Asn	Asn	Glu	Ser	Glu	Ile	Ile	
1				5					10					15		
Glu	Ser	Phe	Ile	Arg	Tyr	Asn	Tyr	Asn	Phe	Ile	Asp	Glu	Met	Val	Ile	
			20					25					30			
Ile	Asp	Asn	Gly	Cys	Thr	Asp	Asn	Thr	Met	Gln	Ile	Ile	Phe	Asn	Leu	
		35					40					45				
Ile	Lys	Glu	Gly	Tyr	Lys	Ile	Ser	Val	Tyr	Asp	Glu	Ser	Leu	Glu	Ala	
	50					55					60					
Tyr	Asn	Gln	Tyr	Arg	Leu	Asp	Asn	Lys	Tyr	Leu	Thr	Lys	Ile	Ile	Ala	
65					70					75					80	
Glu	Lys	Asn	Pro	Asp	Leu	Ile	Ile	Pro	Leu	Asp	Ala	Asp	Glu	Phe	Leu	
				85					90					95		
Thr	Ala	Asp	Ser	Asn	Pro	Arg	Lys	Leu	Leu	Glu	Gln	Leu	Asp	Leu	Glu	
			100					105					110			
Lys	Ile	His	Tyr	Val	Asn	Trp	Gln	Trp	Phe	Val	Met	Thr	Lys	Lys	Asp	
		115					120					125				
Asp	Ile	Asn	Asp	Ser	Phe	Ile	Pro	Arg	Arg	Met	Gln	Tyr	Cys	Phe	Glu	
	130					135					140					
Lys	Pro	Val	Trp	His	His	Ser	Asp	Gly	Lys	Pro	Val	Thr	Lys	Cys	Ile	
145					150					155					160	
Ile	Ser	Ala	Lys	Tyr	Tyr	Lys	Lys	Met	Asn	Leu	Lys	Leu	Ser	Met	Gly	
				165					170					175		
His	His	Thr	Val	Phe	Gly	Asn	Pro	Asn	Val	Arg	Ile	Glu	His	His	Asn	
			180					185					190			
Asp	Leu	Lys	Phe	Ala	His	Tyr	Arg	Ala	Ile	Ser	Gln	Glu	Gln	Leu	Ile	
		195					200					205				
Tyr	Lys	Thr	Ile	Cys	Tyr	Thr	Ile	Arg	Asp	Ile	Ala	Thr	Met	Glu	Asn	
	210					215					220					
Asn	Ile	Glu	Thr	Ala	Gln	Arg	Thr	Asn	Gln	Met	Ala	Leu	Ile	Glu	Ser	
225					230					235					240	
Gly	Val	Asp	Met	Trp	Glu	Thr	Ala	Arg	Glu	Ala	Ser	Tyr	Ser	Gly	Tyr	
				245					250					255		

Asp Cys Asn Val Ile His Ala Pro Ile Asp Leu Ser Phe Cys Lys Glu
 260 265 270
 Asn Ile Val Ile Lys Tyr Asn Glu Leu Ser Arg Glu Thr Val Ala Glu
 275 280 285
 Arg Val Met Lys Thr Gly Arg Glu Met Ala Val Arg Ala Tyr Asn Val
 290 295 300
 Glu Arg Lys Gln Lys Glu Lys Lys Phe Leu Lys Pro Ile Ile Phe Val
 305 310 315 320
 Leu Asp Gly Leu Lys Gly Asp Glu Tyr Ile His Pro Asn Pro Ser Asn
 325 330 335
 His Leu Thr Ile Leu Thr Glu Met Tyr Asn Val Arg Gly Leu Leu Thr
 340 345 350
 Asp Asn His Gln Ile Lys Phe Leu Lys Val Asn Tyr Arg Leu Ile Ile
 355 360 365
 Thr Pro Asp Phe Ala Lys Phe Leu Pro His Glu Phe Ile Val Val Pro
 370 375 380
 Asp Thr Leu Asp Ile Glu Gln Val Lys Ser Gln Tyr Val Gly Thr Gly
 385 390 395 400
 Val Asp Leu Ser Lys Ile Ile Ser Leu Lys Glu Tyr Arg Lys Glu Ile
 405 410 415
 Gly Phe Ile Gly Asn Leu Tyr Ala Leu Leu Gly Phe Val Pro Asn Met
 420 425 430
 Leu Asn Arg Ile Tyr Leu Tyr Ile Gln Arg Asn Gly Ile Ala Asn Thr
 435 440 445
 Ile Ile Lys Ile Lys Ser Arg Leu
 450 455

<210> 20

<211> 410

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2I

<400> 20

Met	Gln	Ala	Asp	Arg	Arg	Lys	Thr	Phe	Gly	Lys	Met	Arg	Ile	Arg	Ile	
1				5					10					15		
Asn	Asn	Leu	Phe	Phe	Val	Ala	Ile	Ala	Phe	Met	Gly	Ile	Ile	Ile	Ser	
			20					25					30			
Asn	Ser	Gln	Val	Val	Leu	Ala	Ile	Gly	Lys	Ala	Ser	Val	Ile	Gln	Tyr	
		35					40					45				
Leu	Ser	Tyr	Leu	Val	Leu	Ile	Leu	Cys	Ile	Val	Asn	Asp	Leu	Leu	Lys	
	50					55					60					
Asn	Asn	Lys	His	Ile	Val	Val	Tyr	Lys	Leu	Gly	Tyr	Leu	Phe	Leu	Ile	
65					70					75					80	
Ile	Phe	Leu	Phe	Thr	Ile	Gly	Ile	Cys	Gln	Gln	Ile	Leu	Pro	Ile	Thr	
				85					90					95		
Thr	Lys	Ile	Tyr	Leu	Ser	Ile	Ser	Met	Met	Ile	Ile	Ser	Val	Leu	Ala	
			100					105					110			
Thr	Leu	Pro	Ile	Ser	Leu	Ile	Lys	Asp	Ile	Asp	Asp	Phe	Arg	Arg	Ile	
		115					120					125				
Ser	Asn	His	Leu	Leu	Phe	Ala	Leu	Phe	Ile	Thr	Ser	Ile	Leu	Gly	Ile	
	130					135					140					
Lys	Met	Gly	Ala	Thr	Met	Phe	Thr	Gly	Ala	Val	Glu	Gly	Ile	Gly	Phe	
145					150					155					160	
Ser	Gln	Gly	Phe	Asn	Gly	Gly	Leu	Thr	His	Lys	Asn	Phe	Phe	Gly	Ile	
				165					170					175		
Thr	Ile	Leu	Met	Gly	Phe	Val	Leu	Thr	Tyr	Leu	Ala	Tyr	Lys	Tyr	Gly	
		180						185					190			
Ser	Tyr	Lys	Arg	Thr	Asp	Arg	Phe	Ile	Leu	Gly	Leu	Glu	Leu	Phe	Leu	
		195					200					205				
Ile	Leu	Ile	Ser	Asn	Thr	Arg	Ser	Val	Tyr	Leu	Ile	Leu	Leu	Leu	Phe	
	210					215					220					
Leu	Phe	Leu	Val	Asn	Leu	Asp	Lys	Ile	Lys	Ile	Glu	Gln	Arg	Gln	Trp	
225					230					235					240	
Ser	Thr	Leu	Lys	Tyr	Ile	Ser	Met	Leu	Phe	Cys	Ala	Ile	Phe	Leu	Tyr	
				245					250					255		

Tyr Phe Phe Gly Phe Leu Ile Thr His Ser Asp Ser Tyr Ala His Arg
 260 265 270
 Val Asn Gly Leu Ile Asn Phe Phe Glu Tyr Tyr Arg Asn Asp Trp Phe
 275 280 285
 His Leu Met Phe Gly Ala Ala Asp Leu Ala Tyr Gly Asp Leu Thr Leu
 290 295 300
 Asp Tyr Ala Ile Arg Val Arg Arg Val Leu Gly Trp Asn Gly Thr Leu
 305 310 315 320
 Glu Met Pro Leu Leu Ser Ile Met Leu Lys Asn Gly Phe Ile Gly Leu
 325 330 335
 Val Gly Tyr Gly Ile Val Leu Tyr Lys Leu Tyr Arg Asn Val Arg Ile
 340 345 350
 Leu Lys Thr Asp Asn Ile Lys Thr Ile Gly Lys Ser Val Phe Ile Ile
 355 360 365
 Val Val Leu Ser Ala Thr Val Glu Asn Tyr Ile Val Asn Leu Ser Phe
 370 375 380
 Val Phe Met Pro Ile Cys Phe Cys Leu Leu Asn Ser Ile Ser Thr Met
 385 390 395 400
 Glu Ser Thr Ile Asn Lys Gln Leu Gln Thr
 405 410

<210> 21

<211> 332

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2J

<400> 21

Met Glu Lys Val Ser Ile Ile Val Pro Ile Phe Asn Thr Glu Lys Tyr
 1 5 10 15
 Leu Arg Glu Cys Leu Asp Ser Ile Ile Ser Gln Ser Tyr Thr Asn Leu
 20 25 30

Glu	Ile	Leu	Leu	Ile	Asp	Asp	Gly	Ser	Ser	Asp	Ser	Ser	Thr	Asp	Ile	35	40	45
Cys	Leu	Glu	Tyr	Ala	Glu	Gln	Asp	Gly	Arg	Ile	Lys	Leu	Phe	Arg	Leu	50	55	60
Pro	Asn	Gly	Gly	Val	Ser	Asn	Ala	Arg	Asn	Tyr	Gly	Ile	Lys	Asn	Ser	65	70	75
Thr	Ala	Asn	Tyr	Ile	Met	Phe	Val	Asp	Ser	Asp	Asp	Ile	Val	Asp	Gly	85	90	95
Asn	Ile	Val	Glu	Ser	Leu	Tyr	Thr	Cys	Leu	Lys	Glu	Asn	Asp	Ser	Asp	100	105	110
Leu	Ser	Gly	Gly	Leu	Leu	Ala	Thr	Phe	Asp	Gly	Asn	Tyr	Gln	Glu	Ser	115	120	125
Glu	Leu	Gln	Lys	Cys	Gln	Ile	Asp	Leu	Glu	Glu	Ile	Lys	Glu	Val	Arg	130	135	140
Asp	Leu	Gly	Asn	Glu	Asn	Phe	Pro	Asn	His	Tyr	Met	Ser	Gly	Ile	Phe	145	150	155
Asn	Ser	Pro	Cys	Cys	Lys	Leu	Tyr	Lys	Asn	Ile	Tyr	Ile	Asn	Gln	Gly	165	170	175
Phe	Asp	Thr	Glu	Gln	Trp	Leu	Gly	Glu	Asp	Leu	Leu	Phe	Asn	Leu	Asn	180	185	190
Tyr	Leu	Lys	Asn	Ile	Lys	Lys	Val	Arg	Tyr	Val	Asn	Arg	Asn	Leu	Tyr	195	200	205
Phe	Ala	Arg	Arg	Ser	Leu	Gln	Ser	Thr	Thr	Asn	Thr	Phe	Lys	Tyr	Asp	210	215	220
Val	Phe	Ile	Gln	Leu	Glu	Asn	Leu	Glu	Glu	Lys	Thr	Phe	Asp	Leu	Phe	225	230	235
Val	Lys	Ile	Phe	Gly	Gly	Gln	Tyr	Glu	Phe	Ser	Val	Phe	Lys	Glu	Thr	245	250	255
Leu	Gln	Trp	His	Ile	Ile	Tyr	Tyr	Ser	Leu	Leu	Met	Phe	Lys	Asn	Gly	260	265	270
Asp	Glu	Ser	Leu	Pro	Lys	Lys	Leu	His	Ile	Phe	Lys	Tyr	Leu	Tyr	Asn	275	280	285
Arg	His	Ser	Leu	Asp	Thr	Leu	Ser	Ile	Lys	Arg	Thr	Ser	Ser	Val	Phe	290	295	300

Lys Arg Ile Cys Lys Leu Ile Val Ala Asn Asn Leu Phe Lys Ile Phe
 305 310 315 320

Leu Asn Thr Leu Ile Arg Glu Glu Lys Asn Asn Asp
 325 330

<210> 22

<211> 332

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2K

<400> 22

Met Ile Asn Ile Ser Ile Ile Val Pro Ile Tyr Asn Val Glu Gln Tyr
 1 5 10 15

Leu Ser Lys Cys Ile Asn Ser Ile Val Asn Gln Thr Tyr Lys His Ile
 20 25 30

Glu Ile Leu Leu Val Asn Asp Gly Ser Thr Asp Asn Ser Glu Glu Ile
 35 40 45

Cys Leu Ala Tyr Ala Lys Lys Asp Ser Arg Ile Arg Tyr Phe Lys Lys
 50 55 60

Glu Asn Gly Gly Leu Ser Asp Ala Arg Asn Tyr Gly Ile Ser Arg Ala
 65 70 75 80

Lys Gly Asp Tyr Leu Ala Phe Ile Asp Ser Asp Asp Phe Ile His Ser
 85 90 95

Glu Phe Ile Gln Arg Leu His Glu Ala Ile Glu Arg Glu Asn Ala Leu
 100 105 110

Val Ala Val Ala Gly Tyr Asp Arg Val Asp Ala Ser Gly His Phe Leu
 115 120 125

Thr Ala Glu Pro Leu Pro Thr Asn Gln Ala Val Leu Ser Gly Arg Asn
 130 135 140

Val Cys Lys Lys Leu Leu Glu Ala Asp Gly His Arg Phe Val Val Ala
 145 150 155 160

Trp	Asn	Lys	Leu	Tyr	Lys	Lys	Glu	Leu	Phe	Asp	Phe	Arg	Phe	Glu	Lys	
				165					170					175		
Gly	Lys	Ile	His	Glu	Asp	Glu	Tyr	Phe	Thr	Tyr	Arg	Leu	Leu	Tyr	Glu	
			180					185					190			
Leu	Glu	Lys	Val	Ala	Ile	Val	Lys	Glu	Cys	Leu	Tyr	Tyr	Tyr	Val	Asp	
		195					200					205				
Arg	Glu	Asn	Ser	Ile	Ile	Thr	Ser	Ser	Met	Thr	Asp	His	Arg	Phe	His	
	210					215					220					
Cys	Leu	Leu	Glu	Phe	Gln	Asn	Glu	Arg	Met	Asp	Phe	Tyr	Glu	Ser	Arg	
225					230					235					240	
Gly	Asp	Lys	Glu	Leu	Leu	Leu	Glu	Cys	Tyr	Arg	Ser	Phe	Leu	Ala	Phe	
				245					250					255		
Ala	Val	Leu	Phe	Leu	Gly	Lys	Tyr	Asn	His	Trp	Leu	Ser	Lys	Gln	Gln	
			260					265					270			
Lys	Lys	Leu	Gln	Thr	Leu	Phe	Arg	Ile	Val	Tyr	Lys	Gln	Leu	Lys	Gln	
		275					280					285				
Asn	Lys	Arg	Leu	Ala	Leu	Leu	Met	Asn	Ala	Tyr	Tyr	Leu	Val	Gly	Cys	
	290					295					300					
Leu	His	Leu	Asn	Phe	Ser	Val	Phe	Leu	Lys	Thr	Gly	Lys	Asp	Lys	Ile	
305					310					315					320	
Gln	Glu	Arg	Leu	Arg	Arg	Ser	Glu	Ser	Ser	Thr	Arg					
				325					330							

<210> 23

<211> 467

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS20

<220>

<221> misc_feature

<222> (1) .. (467)

<223> Xaa may be any amino acid

<400> 23

Met	Ser	Lys	Lys	Ser	Ile	Val	Val	Ser	Gly	Leu	Val	Tyr	Thr	Ile	Gly	
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Thr	Ile	Leu	Val	Gln	Gly	Leu	Ala	Phe	Ile	Thr	Leu	Pro	Ile	Tyr	Thr	
			20					25					30			
Arg	Val	Ile	Ser	Gln	Glu	Val	Tyr	Gly	Gln	Phe	Ser	Leu	Tyr	Asn	Ser	
		35					40					45				
Trp	Val	Gly	Leu	Val	Gly	Leu	Phe	Ile	Gly	Leu	Gln	Leu	Gly	Gly	Ala	
	50					55					60					
Phe	Gly	Pro	Gly	Trp	Val	His	Phe	Arg	Glu	Lys	Phe	Asp	Asp	Phe	Val	
65					70					75					80	
Ser	Thr	Leu	Met	Val	Ser	Ser	Ile	Ala	Phe	Phe	Leu	Pro	Ile	Phe	Gly	
				85					90					95		
Leu	Ser	Phe	Leu	Leu	Ser	Gln	Pro	Leu	Ser	Leu	Leu	Phe	Gly	Leu	Pro	
			100					105					110			
Asp	Trp	Val	Val	Pro	Leu	Ile	Phe	Leu	Gln	Ser	Leu	Met	Ile	Val	Val	
		115					120					125				
Gln	Gly	Phe	Phe	Thr	Thr	Tyr	Leu	Val	Gln	Arg	Gln	Gln	Ser	Met	Trp	
	130					135					140					
Thr	Leu	Pro	Leu	Ser	Val	Leu	Ser	Ala	Val	Ile	Asn	Thr	Ala	Leu	Ser	
145					150					155					160	
Leu	Phe	Leu	Thr	Phe	Pro	Met	Glu	Asn	Asp	Phe	Ile	Ala	Arg	Val	Met	
				165					170					175		
Ala	Asn	Pro	Ala	Thr	Thr	Gly	Val	Leu	Ala	Cys	Val	Ser	Xaa	Trp	Phe	
			180					185					190			
Ser	Gln	Lys	Lys	Asn	Gly	Leu	His	Phe	Arg	Lys	Asp	Tyr	Leu	Arg	Tyr	
		195				200						205				
Gly	Leu	Ser	Ile	Ser	Ile	Pro	Leu	Ile	Phe	His	Gly	Leu	Gly	His	Asn	
	210					215					220					
Val	Leu	Asn	Gln	Phe	Asp	Arg	Ile	Met	Leu	Gly	Lys	Met	Leu	Thr	Leu	
225					230					235					240	

Ser Asp Val Ala Leu Tyr Ser Phe Gly Tyr Thr Leu Ala Ser Ile Leu
 245 250 255
 Gln Ile Val Phe Ser Ser Leu Asn Thr Val Trp Cys Pro Trp Tyr Phe
 260 265 270
 Glu Lys Lys Arg Gly Ala Asp Lys Asp Leu Leu Ser Tyr Val Arg Tyr
 275 280 285
 Tyr Leu Ala Ile Gly Leu Phe Val Thr Phe Gly Phe Leu Thr Ile Tyr
 290 295 300
 Pro Arg Leu Ala Met Leu Leu Gly Gly Ser Glu Tyr Arg Phe Ser Met
 305 310 315 320
 Gly Phe Ile Pro Met Ile Ile Val Gly Val Phe Phe Val Phe Leu Tyr
 325 330 335
 Ser Phe Pro Ala Asn Ile Gln Phe Tyr Ser Gly Asn Thr Lys Phe Leu
 340 345 350
 Pro Ile Gly Thr Phe Ile Ala Gly Val Leu Asn Ile Ser Val His Phe
 355 360 365
 Val Leu Ile Pro Thr Lys Asn Leu Trp Cys Cys Phe Ala Thr Thr Ala
 370 375 380
 Ser Tyr Leu Leu Leu Leu Val Leu His Tyr Phe Val Ala Lys Lys Lys
 385 390 395 400
 Tyr Ala Tyr Asp Glu Val Ala Ile Ser Thr Phe Val Lys Val Ile Ala
 405 410 415
 Leu Val Val Val Tyr Thr Gly Leu Met Thr Val Phe Val Gly Ser Ile
 420 425 430
 Trp Ile Arg Trp Ser Leu Gly Ile Ala Val Leu Val Val Tyr Ala Ile
 435 440 445
 Tyr Phe Arg Lys Glu Leu Thr Val Ala Leu Asn Thr Phe Arg Glu Lys
 450 455 460
 Arg Ser Lys
 465

<210> 24

<211> 338

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2P

<400> 24

Met	Val	Tyr	Ile	Ile	Ala	Glu	Ile	Gly	Cys	Asn	His	Asn	Gly	Asp	Val	
1				5					10					15		
His	Leu	Ala	Arg	Lys	Met	Val	Glu	Val	Ala	Val	Asp	Cys	Gly	Val	Asp	
			20					25					30			
Ala	Val	Lys	Phe	Gln	Thr	Glu	Lys	Ala	Asp	Leu	Leu	Ile	Ser	Lys	Tyr	
		35					40					45				
Ala	Pro	Lys	Ala	Glu	Tyr	Gln	Lys	Ile	Thr	Thr	Gly	Glu	Ser	Asp	Ser	
	50					55					60					
Gln	Leu	Glu	Met	Thr	Arg	Arg	Leu	Glu	Leu	Ser	Phe	Glu	Glu	Tyr	Leu	
65					70					75					80	
Asp	Leu	Arg	Asp	Tyr	Cys	Leu	Glu	Lys	Gly	Val	Asp	Val	Phe	Ser	Thr	
				85					90					95		
Pro	Glu	Asp	Glu	Glu	Ser	Leu	Asp	Phe	Leu	Ile	Ser	Thr	Asp	Met	Pro	
			100					105					110			
Val	Tyr	Lys	Ile	Pro	Ser	Gly	Glu	Ile	Thr	Asn	Leu	Pro	Tyr	Leu	Glu	
		115					120					125				
Lys	Ile	Gly	Arg	Gln	Ala	Lys	Lys	Val	Ile	Leu	Ser	Thr	Gly	Met	Ala	
	130					135					140					
Val	Met	Asp	Glu	Ile	His	Gln	Ala	Val	Lys	Ile	Leu	Gln	Glu	Asn	Gly	
145					150					155					160	
Thr	Thr	Asp	Ile	Ser	Ile	Leu	His	Cys	Thr	Thr	Glu	Tyr	Pro	Thr	Pro	
				165					170					175		
Tyr	Pro	Ala	Leu	Asn	Leu	Asn	Val	Leu	His	Thr	Leu	Lys	Lys	Glu	Phe	
			180					185					190			
Pro	Asn	Leu	Thr	Ile	Gly	Tyr	Ser	Asp	His	Ser	Val	Gly	Ser	Glu	Val	
		195					200					205				
Pro	Ile	Ala	Ala	Ala	Ala	Met	Gly	Ala	Glu	Leu	Ile	Glu	Lys	His	Phe	
	210					215					220					

Thr	Leu	Asp	Asn	Glu	Met	Glu	Gly	Pro	Asp	His	Lys	Ala	Ser	Ala	Thr
225					230					235					240
Pro	Asp	Ile	Leu	Ala	Ala	Leu	Val	Lys	Gly	Val	Arg	Ile	Val	Glu	Gln
				245					250					255	
Ser	Leu	Gly	Lys	Phe	Glu	Lys	Glu	Pro	Glu	Glu	Val	Glu	Val	Arg	Asn
			260					265					270		
Lys	Ile	Val	Ala	Glu	Lys	Ser	Ile	Val	Ala	Lys	Lys	Ala	Ile	Ala	Lys
		275					280					285			
Gly	Glu	Val	Phe	Thr	Glu	Glu	Asn	Ile	Thr	Val	Lys	Arg	Pro	Gly	Asn
	290						295				300				
Gly	Ile	Ser	Pro	Met	Glu	Trp	Tyr	Lys	Val	Leu	Gly	Gln	Val	Ser	Glu
305					310					315					320
Gln	Asp	Phe	Glu	Glu	Asp	Gln	Asn	Ile	Cys	His	Ser	Ala	Phe	Glu	Asn
				325					330					335	

Gln Met

<210> 25

<211> 170

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2Q

<400> 25

Met	Lys	Lys	Ile	Cys	Phe	Val	Thr	Gly	Ser	Arg	Ala	Glu	Tyr	Gly	Ile
1				5					10					15	
Met	Arg	Arg	Leu	Leu	Ser	Tyr	Leu	Gln	Asp	Asp	Pro	Glu	Met	Glu	Leu
			20					25					30		
Asp	Leu	Val	Val	Ala	Thr	Met	His	Leu	Glu	Glu	Lys	Tyr	Gly	Met	Thr
		35					40					45			
Val	Lys	Asp	Ile	Glu	Ala	Asp	Lys	Arg	Arg	Ile	Val	Lys	Arg	Ile	Pro
	50					55					60				

Leu His Leu Thr Asp Thr Ser Lys Gln Thr Ile Val Lys Ser Leu Ala
 65 70 75 80
 Thr Leu Thr Glu Gln Leu Thr Val Leu Phe Glu Glu Val Gln Tyr Asp
 85 90 95
 Leu Val Leu Ile Leu Gly Asp Arg Tyr Glu Met Leu Pro Val Ala Asn
 100 105 110
 Ala Ala Leu Leu Tyr Asn Ile Pro Ile Cys His Ile His Gly Gly Glu
 115 120 125
 Lys Thr Met Gly Asn Phe Asp Glu Ser Ile Arg His Ala Ile Thr Lys
 130 135 140
 Met Ser His Leu His Leu Thr Ser Thr Asp Glu Phe Arg Asn Arg Val
 145 150 155 160
 Ile Gln Leu Gly Glu Asn Pro Thr Met Tyr
 165 170

<210> 26

<211> 184

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2R

<400> 26

Met Glu Leu Gly Ile Asp Phe Ala Glu Asp Tyr Tyr Val Val Leu Phe
 1 5 10 15
 His Pro Val Thr Leu Glu Asp Asn Thr Ala Glu Glu Gln Thr Gln Ala
 20 25 30
 Leu Leu Asp Ala Leu Lys Glu Asp Gly Ser Gln Cys Leu Ile Ile Gly
 35 40 45
 Ser Asn Ser Asp Thr His Ala Asp Lys Ile Met Glu Leu Met His Glu
 50 55 60

Phe Val Lys Gln Asp Ser Asp Ser Tyr Ile Phe Thr Ser Leu Pro Thr
 65 70 75 80
 Arg Tyr Tyr His Ser Leu Val Lys His Ser Gln Gly Leu Ile Gly Asn
 85 90 95
 Ser Ser Ser Gly Leu Ile Glu Val Pro Ser Leu Gln Val Pro Thr Leu
 100 105 110
 Asn Ile Gly Asn Arg Gln Phe Gly Arg Leu Ser Gly Pro Ser Val Val
 115 120 125
 His Val Gly Thr Ser Lys Glu Ala Ile Val Gly Gly Leu Gly Gln Leu
 130 135 140
 Arg Asp Val Ile Asp Phe Thr Asn Pro Phe Glu Gln Pro Asp Ser Ala
 145 150 155 160
 Leu Gln Gly Tyr Arg Ala Ile Lys Glu Phe Leu Ser Val Gln Ala Ser
 165 170 175
 Thr Met Lys Glu Phe Tyr Asp Arg
 180

<210> 27

<211> 208

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2S

<400> 27

Met Lys Lys Val Ala Phe Leu Gly Ala Gly Thr Phe Ser Asp Gly Val
 1 5 10 15
 Leu Pro Trp Leu Asp Arg Thr Arg Tyr Glu Leu Ile Gly Tyr Phe Glu
 20 25 30
 Asp Lys Pro Ile Ser Asp Tyr Arg Gly Tyr Pro Val Phe Gly Pro Leu
 35 40 45
 Gln Asp Val Leu Thr Tyr Leu Asp Asp Gly Lys Val Asp Ala Val Phe
 50 55 60

Val	Thr	Ile	Gly	Asp	Asn	Val	Lys	Arg	Lys	Glu	Ile	Phe	Asp	Leu	Leu
65					70					75					80
Ala	Lys	Asp	His	Tyr	Asp	Ala	Leu	Phe	Asn	Ile	Ile	Ser	Glu	Gln	Ala
			85						90					95	
Asn	Ile	Phe	Ser	Pro	Asp	Ser	Ile	Lys	Gly	Arg	Gly	Val	Phe	Ile	Gly
			100					105					110		
Phe	Ser	Ser	Phe	Val	Gly	Ala	Asp	Ser	Tyr	Val	Tyr	Asp	Asn	Cys	Ile
		115					120					125			
Ile	Asn	Thr	Gly	Ala	Ile	Val	Glu	His	His	Thr	Thr	Val	Glu	Ala	His
	130					135					140				
Cys	Asn	Ile	Thr	Pro	Gly	Val	Thr	Ile	Asn	Gly	Leu	Cys	Arg	Ile	Gly
145					150					155					160
Glu	Ser	Thr	Tyr	Ile	Gly	Ser	Gly	Ser	Thr	Val	Ile	Gln	Cys	Ile	Glu
				165					170					175	
Ile	Ala	Pro	Tyr	Thr	Thr	Leu	Gly	Ala	Gly	Thr	Val	Val	Leu	Lys	Ser
			180					185					190		
Leu	Thr	Glu	Ser	Gly	Thr	Tyr	Val	Gly	Val	Pro	Ala	Arg	Lys	Ile	Lys
		195					200					205			

<210> 28

<211> 410

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2T

<400> 28

Met	Glu	Pro	Ile	Cys	Leu	Ile	Pro	Ala	Arg	Ser	Gly	Ser	Lys	Gly	Leu
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Pro	Asn	Lys	Asn	Met	Leu	Phe	Leu	Asp	Gly	Val	Pro	Met	Ile	Phe	His
			20					25					30		

Thr	Ile	Arg	Ala	Ala	Ile	Glu	Ser	Gly	Cys	Phe	Lys	Lys	Glu	Asn	Ile		
		35					40					45					
Tyr	Val	Ser	Thr	Asp	Ser	Glu	Val	Tyr	Lys	Glu	Ile	Cys	Glu	Thr	Thr		
	50					55					60						
Gly	Val	Gln	Val	Leu	Met	Arg	Pro	Ala	Asp	Leu	Ala	Thr	Asp	Phe	Thr		
65					70					75					80		
Thr	Ser	Phe	Gln	Leu	Asn	Glu	His	Phe	Leu	Gln	Asp	Phe	Ser	Asp	Asp		
				85					90					95			
Gln	Val	Phe	Val	Leu	Leu	Gln	Val	Thr	Ser	Pro	Leu	Arg	Ser	Gly	Lys		
			100					105					110				
His	Val	Lys	Glu	Ala	Met	Glu	Leu	Tyr	Gly	Lys	Gly	Gln	Ala	Asp	His		
		115					120					125					
Val	Val	Ser	Phe	Thr	Lys	Val	Asp	Lys	Ser	Pro	Thr	Leu	Phe	Ser	Thr		
	130					135					140						
Leu	Asp	Glu	Asn	Gly	Phe	Ala	Lys	Asp	Ile	Ala	Gly	Leu	Gly	Gly	Ser		
145					150					155					160		
Tyr	Arg	Arg	Gln	Asp	Glu	Lys	Thr	Leu	Tyr	Tyr	Pro	Asn	Gly	Ala	Ile		
				165					170					175			
Tyr	Ile	Ser	Ser	Lys	Gln	Ala	Tyr	Leu	Ala	Asp	Lys	Thr	Tyr	Phe	Ser		
			180					185					190				
Glu	Lys	Thr	Ala	Ala	Tyr	Val	Met	Thr	Lys	Glu	Asp	Ser	Ile	Asp	Val		
		195					200					205					
Asp	Asp	His	Phe	Asp	Phe	Thr	Gly	Val	Ile	Gly	Arg	Ile	Tyr	Phe	Asp		
	210					215					220						
Tyr	Gln	Arg	Arg	Glu	Gln	Gln	Asn	Lys	Pro	Phe	Tyr	Lys	Arg	Glu	Leu		
225					230					235					240		
Lys	Arg	Leu	Cys	Glu	Gln	Arg	Val	His	Asp	Ser	Leu	Val	Ile	Gly	Asp		
				245				250						255			
Ser	Arg	Leu	Leu	Ala	Leu	Leu	Leu	Asp	Gly	Phe	Asp	Asn	Ile	Ser	Ile		
			260				265						270				
Gly	Gly	Met	Thr	Ala	Ser	Thr	Ser	Leu	Glu	Asn	Gln	Gly	Leu	Phe	Leu		
		275					280					285					
Ala	Thr	Pro	Ile	Lys	Lys	Val	Leu	Leu	Ser	Leu	Gly	Val	Asn	Asp	Leu		
	290					295					300						

Ile	Thr	Asp	Tyr	Pro	Leu	His	Met	Ile	Glu	Asp	Thr	Ile	Arg	Gln	Leu
305					310					315					320
Met	Glu	Ser	Leu	Val	Ser	Lys	Ala	Glu	Gln	Val	Glu	Val	Thr	Thr	Ile
			325						330					335	
Ala	Tyr	Thr	Leu	Phe	Arg	Asp	Ser	Val	Ser	Asn	Glu	Glu	Thr	Val	Gln
			340					345					350		
Leu	Asn	Asp	Val	Ile	Val	Gln	Ser	Ala	Ser	Glu	Leu	Gly	Ile	Ser	Val
		355					360					365			
Ile	Asp	Leu	Asn	Glu	Val	Val	Glu	Lys	Glu	Ala	Met	Leu	Asp	Tyr	Gln
	370					375					380				
Tyr	Thr	Asn	Asp	Gly	Leu	His	Phe	Asn	Gln	Ile	Gly	Gln	Glu	Arg	Val
385					390					395					400
Asn	Gln	Leu	Ile	Leu	Thr	Ser	Leu	Thr	Arg						
			405						410						

<210> 29

<211> 6992

<212> DNA

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1

<400> 29

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caagtcatat accaaatgct gatttaaadc gttctggaat ttttatcata atgatggttc	120
attattttgc attttttata tctcgtatgc cagttgaatt tgagtataga ggtaaatctga	180
tagagtttga aaaaacattt aactatagta taatatttgc aatttttctt acggcagtat	240
catttttggt ggagaataat ttcgcacttt caagacgtgg tgccgtgtat ttcacattaa	300
taaacttcgt tttggtatac ctatttaacg taattattaa gcagtttaag gatagctttc	360
tattttcgac aatctatcaa aaaaagacga ttctaattac aacggctgaa cgatgggaaa	420

atatgcaagt tttatattgaa tcacataaac aaattcaaaa aaatcttggt gcattggtag 480
 ttttaggtac agaaatagat aaaattaatt tatcattacc gctctattat tctgtggaag 540
 aagctataga gttttcaaca agggaagtgg tcgaccacgt ctttataaat ctaccaagtg 600
 agtttttaga cgtaaagcaa ttctgttcag attttgagtt gttaggtatt gatgtaagcg 660
 ttgatattaa ttcattcggt tttactgcgt tgaaaaacaa aaaaatccaa ctgctaggtg 720
 accatagcat tgtaactttt tccacaaatt tttataagcc tagtcatatc atgatgaaac 780
 gacttttgga tatactcgga gcggtagtcg ggttaattat ttgtggtata gtttctattt 840
 tgtagttcc aattattcgt agagatgggt gaccggctat ttttgctcag aaacgagttg 900
 gacagaatgg acgcatattt acattctaca agtttcgac gatgtatgtt gatgctgagg 960
 agcgcaaaaa agacttgctc agccaaaacc agatgcaagg gtgggtatgt tttaaaatgg 1020
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 agttaccaca gttttataat gttttaattg gcgatatgag tctagttggg acacgtccac 1140
 ctacagttga tgaatttgaa aaatatactc ctgggtcaaaa gagacgattg agttttaaac 1200
 cagggattac aggtctctgg caggttagtg gtcgtagtaa tatcacagac ttcgacgacg 1260
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 ggaaggaaga agaacgtttt tgggtaacat ttgataaaga ggatgcaaga agtcttttga 1500
 agaatgaaaa aatgtatcca tgttactttc caacaaatcg caatctcatt aatttagtga 1560
 aaaatacttt cttagctttc aaaattttac gtgatgagaa accagatgtt attatttcat 1620
 ctggtgcggc cgttgctgtc cccttctttt acatcggaag actatttgga gcaaagacga 1680
 tttatattga agtatttgat cgagttaata aatctacatt aactggaaaa ctagtttatc 1740
 ccgtaacaga tattttttatt gttcagtggg aagaaatgaa gaaggtatat cctaaatcta 1800
 ttaacttggg gagtattttt taatgatttt tgtaacagta ggaactcatg aacaacagtt 1860
 taatcgattg ataaaagaga ttgatttatt gaaaaaaaaat ggaagtataa ccgacgaaat 1920
 atttattcaa acaggatatt ctgactatat tccagaatat tgcaagtata aaaaatttct 1980

cagttacaaa gaaatggaac aatatattaa caaatcagaa gtagttatTTT gccacggagg 2040
ccccgctact tttatgaatt cattatccaa aggaaaaaaa caattattgt ttcctagaca 2100
aaaaaagtat ggtgaacatg taaatgatca tcaagtagag tttgtaagaa gaattttaca 2160
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<210> 30

<211> 454

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1E

<400> 30

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Ala	Ile	Leu	Thr	Ser	His	Ile	Pro	Asn	Ala	Asp	Leu	Asn	Arg	Ser	Gly	20	25	30	
Ile	Phe	Ile	Ile	Met	Met	Val	His	Tyr	Phe	Ala	Phe	Phe	Ile	Ser	Arg	35	40	45	
Met	Pro	Val	Glu	Phe	Glu	Tyr	Arg	Gly	Asn	Leu	Ile	Glu	Phe	Glu	Lys	50	55	60	
Thr	Phe	Asn	Tyr	Ser	Ile	Ile	Phe	Ala	Ile	Phe	Leu	Thr	Ala	Val	Ser	65	70	75	80
Phe	Leu	Leu	Glu	Asn	Asn	Phe	Ala	Leu	Ser	Arg	Arg	Gly	Ala	Val	Tyr	85	90	95	
Phe	Thr	Leu	Ile	Asn	Phe	Val	Leu	Val	Tyr	Leu	Phe	Asn	Val	Ile	Ile	100	105	110	
Lys	Gln	Phe	Lys	Asp	Ser	Phe	Leu	Phe	Ser	Thr	Ile	Tyr	Gln	Lys	Lys	115	120	125	

Thr	Ile	Leu	Ile	Thr	Thr	Ala	Glu	Arg	Trp	Glu	Asn	Met	Gln	Val	Leu	
	130					135					140					
Phe	Glu	Ser	His	Lys	Gln	Ile	Gln	Lys	Asn	Leu	Val	Ala	Leu	Val	Val	
145					150					155					160	
Leu	Gly	Thr	Glu	Ile	Asp	Lys	Ile	Asn	Leu	Ser	Leu	Pro	Leu	Tyr	Tyr	
				165					170					175		
Ser	Val	Glu	Glu	Ala	Ile	Glu	Phe	Ser	Thr	Arg	Glu	Val	Val	Asp	His	
				180				185					190			
Val	Phe	Ile	Asn	Leu	Pro	Ser	Glu	Phe	Leu	Asp	Val	Lys	Gln	Phe	Val	
		195					200					205				
Ser	Asp	Phe	Glu	Leu	Leu	Gly	Ile	Asp	Val	Ser	Val	Asp	Ile	Asn	Ser	
	210					215					220					
Phe	Gly	Phe	Thr	Ala	Leu	Lys	Asn	Lys	Lys	Ile	Gln	Leu	Leu	Gly	Asp	
225					230					235					240	
His	Ser	Ile	Val	Thr	Phe	Ser	Thr	Asn	Phe	Tyr	Lys	Pro	Ser	His	Ile	
				245					250					255		
Met	Met	Lys	Arg	Leu	Leu	Asp	Ile	Leu	Gly	Ala	Val	Val	Gly	Leu	Ile	
			260					265					270			
Ile	Cys	Gly	Ile	Val	Ser	Ile	Leu	Leu	Val	Pro	Ile	Ile	Arg	Arg	Asp	
		275					280					285				
Gly	Gly	Pro	Ala	Ile	Phe	Ala	Gln	Lys	Arg	Val	Gly	Gln	Asn	Gly	Arg	
	290					295					300					
Ile	Phe	Thr	Phe	Tyr	Lys	Phe	Arg	Ser	Met	Tyr	Val	Asp	Ala	Glu	Glu	
305					310					315					320	
Arg	Lys	Lys	Asp	Leu	Leu	Ser	Gln	Asn	Gln	Met	Gln	Gly	Trp	Val	Cys	
				325					330					335		
Phe	Lys	Met	Gly	Lys	Thr	Ile	Leu	Glu	Leu	Leu	Gln	Leu	Asp	Ile	Ser	
			340					345					350			
Tyr	Ala	Lys	Thr	Ser	Leu	Asp	Glu	Leu	Pro	Gln	Phe	Tyr	Asn	Val	Leu	
		355					360					365				
Ile	Gly	Asp	Met	Ser	Leu	Val	Gly	Thr	Arg	Pro	Pro	Thr	Val	Asp	Glu	
	370					375					380					
Phe	Glu	Lys	Tyr	Thr	Pro	Gly	Gln	Lys	Arg	Arg	Leu	Ser	Phe	Lys	Pro	
385					390					395					400	

Gly Ile Thr Gly Leu Trp Gln Val Ser Gly Arg Ser Asn Ile Thr Asp
 405 410 415
 Phe Asp Asp Val Val Arg Leu Asp Leu Ala Tyr Ile Asp Asn Trp Thr
 420 425 430
 Ile Trp Ser Asp Ile Lys Ile Leu Leu Lys Thr Val Lys Val Val Leu
 435 440 445
 Leu Arg Glu Gly Ser Lys
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<210> 31

<211> 149

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1F

<400> 31

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 Tyr Leu Leu Lys Pro Phe Trp Lys Glu Glu Glu Arg Phe Trp Val Thr
 20 25 30
 Phe Asp Lys Glu Asp Ala Arg Ser Leu Leu Lys Asn Glu Lys Met Tyr
 35 40 45
 Pro Cys Tyr Phe Pro Thr Asn Arg Asn Leu Ile Asn Leu Val Lys Asn
 50 55 60
 Thr Phe Leu Ala Phe Lys Ile Leu Arg Asp Glu Lys Pro Asp Val Ile
 65 70 75 80
 Ile Ser Ser Gly Ala Ala Val Ala Val Pro Phe Phe Tyr Ile Gly Lys
 85 90 95
 Leu Phe Gly Ala Lys Thr Ile Tyr Ile Glu Val Phe Asp Arg Val Asn
 100 105 110
 Lys Ser Thr Leu Thr Gly Lys Leu Val Tyr Pro Val Thr Asp Ile Phe
 115 120 125

Ile	Val	Gln	Trp	Glu	Glu	Met	Lys	Lys	Val	Tyr	Pro	Lys	Ser	Ile	Asn
	130					135					140				

Leu	Gly	Ser	Ile	Phe
145				

<210> 32

<211> 164

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1G

<400> 32

Met	Ile	Phe	Val	Thr	Val	Gly	Thr	His	Glu	Gln	Gln	Phe	Asn	Arg	Leu
1				5					10					15	

Ile	Lys	Glu	Ile	Asp	Leu	Leu	Lys	Lys	Asn	Gly	Ser	Ile	Thr	Asp	Glu
			20					25					30		

Ile	Phe	Ile	Gln	Thr	Gly	Tyr	Ser	Asp	Tyr	Ile	Pro	Glu	Tyr	Cys	Lys
		35					40					45			

Tyr	Lys	Lys	Phe	Leu	Ser	Tyr	Lys	Glu	Met	Glu	Gln	Tyr	Ile	Asn	Lys
	50					55					60				

Ser	Glu	Val	Val	Ile	Cys	His	Gly	Gly	Pro	Ala	Thr	Phe	Met	Asn	Ser
65					70					75					80

Leu	Ser	Lys	Gly	Lys	Lys	Gln	Leu	Leu	Phe	Pro	Arg	Gln	Lys	Lys	Tyr
				85					90					95	

Gly	Glu	His	Val	Asn	Asp	His	Gln	Val	Glu	Phe	Val	Arg	Arg	Ile	Leu
			100				105						110		

Gln	Asp	Asn	Asn	Ile	Leu	Phe	Ile	Glu	Asn	Ile	Asp	Asp	Leu	Phe	Glu
	115						120					125			

Lys	Ile	Ile	Glu	Val	Ser	Lys	Gln	Thr	Asn	Phe	Thr	Ser	Asn	Asn	Asn
	130					135					140				

Phe	Phe	Cys	Glu	Arg	Leu	Lys	Gln	Ile	Val	Glu	Lys	Phe	Asn	Glu	Asp
145					150					155					160

Gln Glu Asn Glu

<210> 33

<211> 388

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1H

<400> 33

Met	Phe	Lys	Leu	Phe	Lys	Tyr	Asp	Pro	Glu	Tyr	Phe	Ile	Phe	Lys	Tyr
1				5					10					15	

Phe	Trp	Leu	Ile	Ile	Phe	Ile	Pro	Glu	Gln	Lys	Tyr	Val	Phe	Leu	Leu
		20					25					30			

Ile	Phe	Met	Asn	Leu	Ile	Leu	Phe	His	Ile	Lys	Phe	Leu	Lys	Thr	Lys
	35					40					45				

Leu	Ile	Leu	Lys	Asn	Glu	Ile	Leu	Leu	Phe	Leu	Leu	Trp	Ser	Ile	Leu
50					55					60					

Cys	Phe	Val	Ser	Val	Val	Thr	Ser	Met	Phe	Val	Glu	Ile	Asn	Phe	Glu
65				70					75						80

Arg	Leu	Phe	Ala	Asp	Phe	Thr	Ala	Pro	Ile	Ile	Trp	Ile	Ile	Ala	Ile
			85					90						95	

Met	Tyr	Tyr	Asn	Leu	Tyr	Ser	Phe	Ile	Asn	Ile	Asp	Tyr	Lys	Lys	Leu
			100				105						110		

Lys	Asn	Ser	Ile	Phe	Phe	Ser	Phe	Leu	Val	Leu	Leu	Gly	Ile	Ser	Ala
	115					120						125			

Leu	Tyr	Ile	Ile	Gln	Asn	Gly	Lys	Asp	Ile	Val	Phe	Leu	Asp	Arg	His
130					135						140				

Leu	Ile	Gly	Leu	Asp	Tyr	Leu	Ile	Thr	Gly	Val	Lys	Thr	Arg	Leu	Val
145				150						155					160

Gly	Phe	Met	Asn	Tyr	Pro	Thr	Leu	Asn	Thr	Thr	Thr	Ile	Ile	Val	Ser		
				165					170					175			
Ile	Pro	Leu	Ile	Phe	Ala	Leu	Ile	Lys	Asn	Lys	Met	Gln	Gln	Phe	Phe		
			180					185					190				
Phe	Leu	Cys	Leu	Ala	Phe	Ile	Pro	Ile	Tyr	Leu	Ser	Gly	Ser	Arg	Ile		
		195					200					205					
Gly	Ser	Leu	Ser	Leu	Ala	Ile	Leu	Ile	Ile	Cys	Leu	Leu	Trp	Arg	Tyr		
	210					215					220						
Ile	Gly	Gly	Lys	Phe	Ala	Trp	Ile	Lys	Lys	Leu	Ile	Val	Ile	Phe	Val		
225					230					235					240		
Ile	Leu	Leu	Ile	Ile	Leu	Asn	Thr	Glu	Leu	Leu	Tyr	His	Glu	Ile	Leu		
				245					250					255			
Ala	Val	Tyr	Asn	Ser	Arg	Glu	Ser	Ser	Asn	Glu	Ala	Arg	Phe	Ile	Ile		
			260					265					270				
Tyr	Gln	Gly	Ser	Ile	Asp	Lys	Val	Leu	Glu	Asn	Asn	Ile	Leu	Phe	Gly		
		275					280					285					
Tyr	Gly	Ile	Ser	Glu	Tyr	Ser	Val	Thr	Gly	Thr	Trp	Leu	Gly	Ser	His		
	290					295					300						
Ser	Gly	Tyr	Ile	Ser	Phe	Phe	Tyr	Lys	Ser	Gly	Ile	Val	Gly	Leu	Ile		
305					310					315					320		
Leu	Leu	Met	Phe	Ser	Phe	Phe	Tyr	Val	Ile	Lys	Lys	Ser	Tyr	Gly	Val		
				325					330					335			
Asn	Gly	Glu	Thr	Ala	Leu	Phe	Tyr	Phe	Thr	Ser	Leu	Ala	Ile	Phe	Phe		
			340					345					350				
Ile	Tyr	Glu	Thr	Ile	Asp	Pro	Ile	Ile	Ile	Ile	Leu	Val	Leu	Phe	Phe		
		355				360						365					
Ser	Ser	Ile	Gly	Ile	Trp	Asn	Asn	Ile	Asn	Phe	Lys	Lys	Asp	Met	Glu		
	370					375					380						
Thr	Lys	Asn	Glu														
385																	

<210> 34

<211> 322

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1I

<400> 34

Met	Asn	Asp	Leu	Ile	Ser	Val	Ile	Val	Pro	Ile	Tyr	Asn	Val	Gln	Asp	
1				5					10					15		
Tyr	Leu	Asp	Lys	Cys	Ile	Asn	Ser	Ile	Ile	Asn	Gln	Thr	Tyr	Thr	Asn	
			20					25					30			
Leu	Glu	Val	Ile	Leu	Val	Asn	Asp	Gly	Ser	Thr	Asp	Asp	Ser	Glu	Lys	
		35					40					45				
Ile	Cys	Leu	Asn	Tyr	Met	Lys	Asn	Asp	Gly	Arg	Ile	Lys	Tyr	Tyr	Lys	
	50					55					60					
Lys	Ile	Asn	Gly	Gly	Leu	Ala	Asp	Ala	Arg	Asn	Phe	Gly	Leu	Glu	His	
65					70					75					80	
Ala	Thr	Gly	Lys	Tyr	Ile	Ala	Phe	Val	Asp	Ser	Asp	Asp	Tyr	Ile	Glu	
			85						90					95		
Val	Ala	Met	Phe	Glu	Arg	Met	His	Asp	Asn	Ile	Thr	Glu	Tyr	Asn	Ala	
		100						105					110			
Asp	Ile	Ala	Glu	Ile	Asp	Phe	Cys	Leu	Val	Asp	Glu	Asn	Gly	Tyr	Thr	
		115					120					125				
Lys	Lys	Lys	Arg	Asn	Ser	Asn	Phe	His	Val	Leu	Thr	Arg	Glu	Glu	Thr	
	130					135					140					
Val	Lys	Glu	Phe	Leu	Ser	Gly	Ser	Asn	Ile	Glu	Asn	Asn	Val	Trp	Cys	
145					150					155					160	
Lys	Leu	Tyr	Ser	Arg	Asp	Ile	Ile	Lys	Asp	Ile	Lys	Phe	Gln	Ile	Asn	
				165					170					175		
Asn	Arg	Ser	Ile	Gly	Glu	Asp	Leu	Leu	Phe	Asn	Leu	Glu	Val	Leu	Asn	
			180					185					190			
Asn	Val	Thr	Arg	Val	Val	Val	Asp	Thr	Arg	Glu	Tyr	Tyr	Tyr	Asn	Tyr	
		195					200					205				
Val	Ile	Arg	Asn	Ser	Ser	Leu	Ile	Asn	Gln	Lys	Phe	Ser	Ile	Asn	Asn	
	210					215					220					

Ile Asp Leu Val Thr Arg Leu Glu Asn Tyr Pro Phe Lys Leu Lys Arg
 225 230 235 240
 Glu Phe Ser His Tyr Phe Asp Ala Lys Val Ile Lys Glu Lys Val Lys
 245 250 255
 Cys Leu Asn Lys Met Tyr Ser Thr Asp Cys Leu Asp Asn Glu Phe Leu
 260 265 270
 Pro Ile Leu Glu Ser Tyr Arg Lys Glu Ile Arg Arg Tyr Pro Phe Ile
 275 280 285
 Lys Ala Lys Arg Tyr Leu Ser Arg Lys His Leu Val Thr Leu Tyr Leu
 290 295 300
 Met Lys Phe Ser Pro Lys Leu Tyr Val Met Leu Tyr Lys Lys Phe Gln
 305 310 315 320
 Lys Gln

<210> 35

<211> 322

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1J

<400> 35

Met Asp Lys Ile Ser Val Ile Val Pro Val Tyr Asn Val Asp Lys Tyr
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 20 25 30
 Glu Ile Leu Leu Ile Asp Asp Gly Ser Val Asp Asp Ser Ala Lys Ile
 35 40 45
 Cys Lys Glu Tyr Glu Lys Asp Lys Arg Val Lys Ile Phe Phe Thr Asn
 50 55 60

His	Ser	Gly	Val	Ser	Asn	Ala	Arg	Asn	His	Gly	Ile	Lys	Arg	Ser	Thr	65	70	75	80
Ala	Glu	Tyr	Ile	Met	Phe	Val	Asp	Ser	Asp	Asp	Val	Val	Asp	Ser	Arg	85	90	95	
Leu	Val	Glu	Lys	Leu	Tyr	Phe	Asn	Ile	Ile	Lys	Ser	Arg	Ser	Asp	Leu	100	105	110	
Ser	Gly	Cys	Leu	Tyr	Ala	Thr	Phe	Ser	Glu	Asn	Ile	Asn	Asn	Phe	Glu	115	120	125	
Val	Asn	Asn	Pro	Asn	Ile	Asp	Phe	Glu	Ala	Ile	Asn	Thr	Val	Gln	Asp	130	135	140	
Met	Gly	Glu	Lys	Asn	Phe	Met	Asn	Leu	Tyr	Ile	Asn	Asn	Ile	Phe	Ser	145	150	155	160
Thr	Pro	Val	Cys	Lys	Leu	Tyr	Lys	Lys	Arg	Tyr	Ile	Thr	Asp	Leu	Phe	165	170	175	
Gln	Glu	Asn	Gln	Trp	Leu	Gly	Glu	Asp	Leu	Leu	Phe	Asn	Leu	His	Tyr	180	185	190	
Leu	Lys	Asn	Ile	Asp	Arg	Val	Ser	Tyr	Leu	Thr	Glu	His	Leu	Tyr	Phe	195	200	205	
Tyr	Arg	Arg	Gly	Ile	Leu	Ser	Thr	Val	Asn	Ser	Phe	Lys	Glu	Gly	Val	210	215	220	
Phe	Leu	Gln	Leu	Glu	Asn	Leu	Gln	Lys	Gln	Val	Ile	Val	Leu	Phe	Lys	225	230	235	240
Gln	Ile	Tyr	Gly	Glu	Asp	Phe	Asp	Val	Ser	Ile	Val	Lys	Asp	Thr	Ile	245	250	255	
Arg	Trp	Gln	Val	Phe	Tyr	Tyr	Ser	Leu	Leu	Met	Phe	Lys	Tyr	Gly	Lys	260	265	270	
Gln	Ser	Ile	Phe	Asp	Lys	Phe	Leu	Ile	Phe	Arg	Asn	Leu	Tyr	Lys	Lys	275	280	285	
Tyr	Tyr	Phe	Asn	Leu	Leu	Lys	Val	Ser	Asn	Lys	Asn	Ser	Leu	Ser	Lys	290	295	300	
Asn	Phe	Cys	Ile	Arg	Ile	Val	Ser	Asn	Lys	Val	Phe	Lys	Lys	Ile	Leu	305	310	315	320
Trp	Leu																		

<210> 36

<211> 278

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS1K

<400> 36

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1 5 10 15

Glu Lys Tyr Leu Ser Lys Cys Ile Asp Ser Ile Val Asn Gln Thr Tyr
20 25 30

Lys His Ile Glu Ile Leu Leu Val Asn Asp Gly Ser Thr Asp Asn Ser
35 40 45

Glu Glu Ile Cys Leu Ala Tyr Ala Lys Lys Asp Ser Arg Ile Arg Tyr
50 55 60

Phe Lys Lys Glu Asn Gly Gly Leu Ser Asp Ala Arg Asn Tyr Gly Ile
65 70 75 80

Ser Arg Ala Lys Gly Asp Tyr Leu Ala Phe Ile Asp Ser Asp Asp Phe
85 90 95

Ile His Ser Glu Phe Ile Gln Arg Leu His Glu Ala Ile Glu Arg Glu
100 105 110

Asn Ala Leu Val Ala Val Ala Gly Tyr Asp Arg Val Asp Ala Ser Gly
115 120 125

His Phe Leu Thr Ala Glu Pro Leu Pro Thr Asn Gln Ala Val Leu Ser
130 135 140

Gly Arg Asn Val Cys Lys Lys Leu Leu Glu Ala Asp Gly His Arg Phe
145 150 155 160

Val Val Ala Cys Asn Lys Leu Tyr Lys Lys Glu Leu Phe Glu Asp Phe
165 170 175

Arg Phe Glu Lys Gly Lys Ile His Glu Asp Glu Tyr Phe Thr Tyr Arg
180 185 190

Leu Leu Tyr Glu Leu Glu Lys Val Ala Ile Val Lys Glu Cys Leu Tyr
 195 200 205
 Tyr Tyr Val Asp Arg Glu Asn Ser Ile Thr Thr Ser Ser Met Thr Asp
 210 215 220
 His Arg Phe His Cys Leu Leu Glu Phe Gln Asn Glu Arg Met Asp Phe
 225 230 235 240
 Tyr Glu Ser Arg Gly Asp Lys Glu Leu Leu Leu Glu Cys Tyr Arg Ser
 245 250 255
 Phe Leu Ala Phe Ala Val Leu Phe Leu Gly Lys Tyr Asn His Trp Leu
 260 265 270
 Ser Lys Gln Gln Lys Lys
 275

<210> 37

<211> 4519

<212> DNA

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS9

<400> 37

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 aagtttatcc tgaaatacga ttgtgctatg gtgctgaatt gtattatagt aaagatatat 180
 taagcaaact tgaaaaaag aaagtaccca cacttaatgg ctgcgctat attcttttgg 240
 agttcagtag tgatactcct tggaaagaga ttcaagaagc agtgaacgaa gtgacgctac 300
 ttgggctaac tcccgtactt gcccatatag aacgatatga cgccctagcg tttcatgcag 360
 agagagtaga agagttaatt gacaagggat gctatactca ggtaaatagt aatcatgtgc 420
 tgaagcccac ttttaattggt gatcgagcaa aagaatttaa aaaacgtact cggtatTTTT 480
 tagagcagga tttagtacat tgtgttgcta gcgatatgca taatttatct agtagacctc 540

cgtttatgag ggaggcttat aagttgctaa cagaggaatt tggcaaagat aaagcgaaag 600
 cgttgctaaa aaagaatcct cttatgctat taaaaaacca ggcgatttaa actggttact 660
 ctagattgtg gagagaaaaa tggatttagg aactgttact gataaactgt tagaacgcaa 720
 cagtaaacga ttgatactcg tgtgcatgga tacgtgtctt cttatagttt ccatgatttt 780
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 ttcattgttt ttaattatct caatgggtgt gtggcaggct tttagttatc gtttcacctt 1020
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 acatgagacg agaaaaaatg ctatccgtaa gaaggatagc ccactaagaa tcttagtagt 1140
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 tgaaattgtc ggtatcggtg atcgatgatcc aaataaactt ggaacattta tccgtacggc 1260
 taaagtttta ggaaaccgta atgatattcc acgactggta gaggaattag ctgttgacca 1320
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 gcatgttcct ttgatggaat ataatccaca tgaagcagtg aagaataata tttttggaac 1860
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 agataaagct gttaatccac caaatgtcat gggagcgact aaacgtgttg cagaaatgat 1980
 tgttacaggt ttaaacgagc caggtcagac tcaatttgcg gcagtcgggt ttgggaatgt 2040
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<210> 38

<211> 215

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS9D

<400> 38

Ala	Tyr	Arg	Gln	Gly	Val	Arg	Tyr	Ile	Val	Ala	Thr	Ser	His	Arg	Arg
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Lys	Gly	Met	Phe	Glu	Thr	Pro	Glu	Lys	Val	Ile	Met	Thr	Asn	Phe	Leu			
			20					25					30					
Gln	Phe	Lys	Asp	Ala	Val	Ala	Glu	Val	Tyr	Pro	Glu	Ile	Arg	Leu	Cys			
		35					40					45						
Tyr	Gly	Ala	Glu	Leu	Tyr	Tyr	Ser	Lys	Asp	Ile	Leu	Ser	Lys	Leu	Glu			
	50					55					60							
Lys	Lys	Lys	Val	Pro	Thr	Leu	Asn	Gly	Ser	Arg	Tyr	Ile	Leu	Leu	Glu			
65					70					75					80			
Phe	Ser	Ser	Asp	Thr	Pro	Trp	Lys	Glu	Ile	Gln	Glu	Ala	Val	Asn	Glu			
				85					90					95				
Val	Thr	Leu	Leu	Gly	Leu	Thr	Pro	Val	Leu	Ala	His	Ile	Glu	Arg	Tyr			
			100					105					110					
Asp	Ala	Leu	Ala	Phe	His	Ala	Glu	Arg	Val	Glu	Glu	Leu	Ile	Asp	Lys			
		115					120					125						
Gly	Cys	Tyr	Thr	Gln	Val	Asn	Ser	Asn	His	Val	Leu	Lys	Pro	Thr	Leu			
	130					135					140							
Ile	Gly	Asp	Arg	Ala	Lys	Glu	Phe	Lys	Lys	Arg	Thr	Arg	Tyr	Phe	Leu			
145					150					155					160			
Glu	Gln	Asp	Leu	Val	His	Cys	Val	Ala	Ser	Asp	Met	His	Asn	Leu	Ser			
				165					170					175				
Ser	Arg	Pro	Pro	Phe	Met	Arg	Glu	Ala	Tyr	Lys	Leu	Leu	Thr	Glu	Glu			
			180					185					190					
Phe	Gly	Lys	Asp	Lys	Ala	Lys	Ala	Leu	Leu	Lys	Lys	Asn	Pro	Leu	Met			
		195					200					205						
Leu	Leu	Lys	Asn	Gln	Ala	Ile												
	210					215												

<210> 39

<211> 608

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS9E

<400> 39

Met	Asp	Leu	Gly	Thr	Val	Thr	Asp	Lys	Leu	Leu	Glu	Arg	Asn	Ser	Lys	
1				5					10					15		
Arg	Leu	Ile	Leu	Val	Cys	Met	Asp	Thr	Cys	Leu	Leu	Ile	Val	Ser	Met	
			20					25					30			
Ile	Leu	Ser	Arg	Leu	Phe	Leu	Asp	Val	Ile	Ile	Asp	Ile	Pro	Asp	Glu	
		35					40					45				
Arg	Phe	Ile	Leu	Ala	Val	Leu	Phe	Val	Ser	Ile	Leu	Tyr	Leu	Ile	Leu	
	50					55					60					
Ser	Phe	Arg	Leu	Lys	Val	Phe	Ser	Leu	Ile	Thr	Arg	Tyr	Thr	Gly	Tyr	
65					70					75					80	
Gln	Ser	Tyr	Val	Lys	Ile	Gly	Leu	Ser	Leu	Ile	Ser	Ala	His	Ser	Leu	
				85					90					95		
Phe	Leu	Ile	Ile	Ser	Met	Val	Leu	Trp	Gln	Ala	Phe	Ser	Tyr	Arg	Phe	
			100					105					110			
Ile	Leu	Val	Ser	Leu	Phe	Leu	Ser	Tyr	Val	Met	Leu	Ile	Thr	Pro	Arg	
		115					120					125				
Ile	Val	Trp	Lys	Val	Leu	His	Glu	Thr	Arg	Lys	Asn	Ala	Ile	Arg	Lys	
	130					135					140					
Lys	Asp	Ser	Pro	Leu	Arg	Ile	Leu	Val	Val	Gly	Ala	Gly	Asp	Gly	Gly	
145					150					155					160	
Asn	Ile	Phe	Ile	Asn	Thr	Val	Lys	Asp	Arg	Lys	Leu	Asn	Phe	Glu	Ile	
				165					170					175		
Val	Gly	Ile	Val	Asp	Arg	Asp	Pro	Asn	Lys	Leu	Gly	Thr	Phe	Ile	Arg	
			180					185					190			
Thr	Ala	Lys	Val	Leu	Gly	Asn	Arg	Asn	Asp	Ile	Pro	Arg	Leu	Val	Glu	
		195					200					205				
Glu	Leu	Ala	Val	Asp	Gln	Val	Thr	Ile	Ala	Ile	Pro	Ser	Leu	Asn	Gly	
	210					215					220					
Lys	Glu	Arg	Glu	Lys	Ile	Val	Glu	Ile	Cys	Asn	Thr	Thr	Gly	Val	Thr	
225					230					235					240	
Val	Asn	Asn	Met	Pro	Ser	Ile	Glu	Asp	Ile	Met	Ala	Gly	Asn	Met	Ser	
				245					250					255		

Val	Ser	Ala	Phe	Gln	Glu	Ile	Asp	Val	Ala	Asp	Leu	Leu	Gly	Arg	Pro		
			260					265					270				
Glu	Val	Val	Leu	Asp	Gln	Asp	Glu	Leu	Asn	Gln	Phe	Phe	Gln	Gly	Lys		
		275					280					285					
Thr	Ile	Leu	Val	Thr	Gly	Ala	Gly	Gly	Ser	Ile	Gly	Ser	Glu	Leu	Cys		
	290					295					300						
Arg	Gln	Ile	Ala	Lys	Phe	Thr	Pro	Lys	Arg	Leu	Leu	Leu	Leu	Gly	His		
305					310					315					320		
Gly	Glu	Asn	Ser	Ile	Tyr	Leu	Ile	His	Arg	Glu	Leu	Leu	Glu	Lys	Tyr		
			325						330					335			
Gln	Gly	Lys	Ile	Glu	Leu	Val	Pro	Leu	Ile	Ala	Asp	Ile	Gln	Asp	Arg		
			340					345					350				
Glu	Leu	Ile	Phe	Ser	Ile	Met	Ala	Glu	Tyr	Gln	Pro	Asp	Val	Val	Tyr		
		355					360					365					
His	Ala	Ala	Ala	His	Lys	His	Val	Pro	Leu	Met	Glu	Tyr	Asn	Pro	His		
	370					375					380						
Glu	Ala	Val	Lys	Asn	Asn	Ile	Phe	Gly	Thr	Lys	Asn	Val	Ala	Glu	Ala		
385					390					395					400		
Ala	Lys	Thr	Ala	Lys	Val	Ala	Lys	Phe	Val	Met	Val	Ser	Thr	Asp	Lys		
				405					410					415			
Ala	Val	Asn	Pro	Pro	Asn	Val	Met	Gly	Ala	Thr	Lys	Arg	Val	Ala	Glu		
			420					425					430				
Met	Ile	Val	Thr	Gly	Leu	Asn	Glu	Pro	Gly	Gln	Thr	Gln	Phe	Ala	Ala		
	435						440					445					
Val	Arg	Phe	Gly	Asn	Val	Leu	Gly	Ser	Arg	Gly	Ser	Val	Val	Pro	Leu		
	450					455					460						
Phe	Lys	Glu	Gln	Ile	Arg	Lys	Gly	Gly	Pro	Val	Thr	Val	Thr	Asp	Phe		
465					470					475					480		
Arg	Met	Thr	Arg	Tyr	Phe	Met	Thr	Ile	Pro	Glu	Ala	Ser	Arg	Leu	Val		
				485					490					495			
Ile	Gln	Ala	Gly	His	Leu	Ala	Lys	Gly	Gly	Glu	Ile	Phe	Val	Leu	Asp		
			500					505					510				
Met	Gly	Glu	Pro	Val	Gln	Ile	Leu	Glu	Leu	Ala	Arg	Lys	Val	Ile	Leu		
		515					520					525					

Leu Ser Gly His Thr Glu Glu Glu Ile Gly Ile Val Glu Ser Gly Ile
530 535 540

Arg Pro Gly Glu Lys Leu Tyr Glu Glu Leu Leu Ser Thr Glu Glu Arg
545 550 555 560

Val Ser Glu Gln Ile His Glu Lys Ile Phe Val Gly Arg Val Thr Asn
565 570 575

Lys Gln Ser Asp Ile Val Asn Ser Phe Ile Asn Gly Leu Leu Gln Lys
580 585 590

Asp Arg Asn Glu Leu Lys Asn Met Leu Ile Glu Phe Ala Lys Gln Glu
595 600 605

<210> 40

<211> 200

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS9F

<400> 40

Met Tyr Pro Ile Cys Lys Arg Ile Leu Ala Ile Ile Ile Ser Gly Ile
1 5 10 15

Ala Ile Val Val Leu Ser Pro Ile Leu Leu Leu Ile Ala Leu Ala Ile
20 25 30

Lys Leu Asp Ser Lys Gly Pro Val Leu Phe Lys Gln Lys Arg Val Gly
35 40 45

Lys Asn Lys Ser Tyr Phe Met Ile Tyr Lys Phe Arg Ser Met Tyr Val
50 55 60

Asp Ala Pro Ser Asp Met Pro Thr His Leu Leu Lys Asp Pro Lys Ala
65 70 75 80

Met Ile Thr Lys Val Gly Ala Phe Leu Arg Lys Thr Ser Leu Asp Glu
85 90 95

Leu Pro Gln Leu Phe Asn Ile Phe Lys Gly Glu Met Ala Ile Val Gly
100 105 110

Pro Arg Pro Ala Leu Trp Asn Gln Tyr Asp Leu Ile Glu Glu Arg Asp
 115 120 125
 Lys Tyr Gly Ala Asn Asp Ile Arg Pro Gly Leu Thr Gly Trp Ala Gln
 130 135 140
 Ile Asn Gly Arg Asp Glu Leu Glu Ile Asp Glu Lys Ser Lys Leu Asp
 145 150 155 160
 Gly Tyr Tyr Val Gln Asn Met Ser Leu Gly Leu Asp Ile Lys Cys Phe
 165 170 175
 Leu Gly Thr Phe Leu Ser Val Ala Arg Ser Glu Gly Val Val Glu Gly
 180 185 190
 Gly Thr Gly Gln Lys Gly Lys Gly
 195 200

<210> 41

<211> 269

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS2G

<400> 41

Met Lys Phe Ser Val Leu Met Ser Val Tyr Glu Lys Glu Lys Pro Glu
 1 5 10 15
 Phe Leu Arg Glu Ser Leu Glu Ser Ile Leu Val Asn Gln Thr Met Ile
 20 25 30
 Pro Thr Glu Val Val Leu Val Glu Asp Gly Pro Leu Asn Gln Ser Leu
 35 40 45
 Tyr Ser Ile Leu Glu Glu Phe Lys Ser Arg Phe Ser Phe Phe Lys Thr
 50 55 60
 Ile Ala Leu Glu Lys Asn Ser Gly Leu Gly Ile Ala Leu Asn Glu Gly
 65 70 75 80

Leu	Lys	His	Cys	Asn	Tyr	Glu	Trp	Val	Cys	Thr	Lys	Trp	Ile	Leu	Met	85	90	95
Met	Leu	His	Ile	His	Thr	Arg	Phe	Glu	Lys	Gln	Val	Asn	Phe	Ile	Lys	100	105	110
Gln	Asn	Pro	Thr	Ile	Asp	Ile	Glu	Ile	Asp	Glu	Phe	Leu	Asn	Ser	Thr	115	120	125
Ser	Glu	Ile	Val	Ser	His	Lys	Asn	Val	Pro	Thr	Gln	His	Asp	Glu	Ile	130	135	140
Leu	Lys	Met	Ala	Arg	Arg	Glu	Lys	Ser	Met	Cys	His	Met	Thr	Val	Met	145	150	155
Phe	Lys	Lys	Lys	Ser	Val	Glu	Arg	Ala	Gly	Gly	Tyr	Gln	Thr	Leu	Pro	165	170	175
Tyr	Val	Glu	Asp	Tyr	Phe	Leu	Trp	Val	Arg	Met	Ile	Ala	Ser	Gly	Ser	180	185	190
Lys	Phe	Ala	Asn	Ile	Asp	Glu	Thr	Leu	Val	Leu	Ala	Arg	Val	Gly	Asn	195	200	205
Gly	Met	Phe	Asn	Arg	Arg	Gly	Asn	Arg	Glu	Gln	Ile	Asn	Ser	Trp	Thr	210	215	220
Leu	Leu	Ile	Glu	Phe	Met	Leu	Ala	Gln	Gly	Ile	Val	Thr	Pro	Leu	Asp	225	230	235
Val	Phe	Ile	Asn	Gln	Ile	Tyr	Ile	Arg	Val	Phe	Val	Tyr	Met	Pro	Thr	245	250	255
Trp	Ile	Lys	Lys	Leu	Ile	Tyr	Gly	Lys	Ile	Leu	Arg	Lys				260	265	

<210> 42

<211> 143

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS9H

<400> 42

Met	Ile	Thr	Val	Leu	Met	Ala	Thr	Tyr	Asn	Gly	Ser	Pro	Phe	Ile	Ile	
1				5					10					15		
Lys	Gln	Leu	Asp	Ser	Ile	Arg	Asn	Gln	Ser	Val	Ser	Ala	Asp	Lys	Val	
			20					25					30			
Ile	Ile	Trp	Asp	Asp	Cys	Ser	Thr	Asp	Asp	Thr	Ile	Lys	Ile	Ile	Lys	
		35					40					45				
Asp	Tyr	Ile	Lys	Lys	Tyr	Ser	Leu	Asp	Ser	Trp	Val	Val	Ser	Gln	Asn	
	50					55					60					
Lys	Ser	Asn	Gln	Gly	His	Tyr	Gln	Thr	Phe	Ile	Asn	Leu	Thr	Lys	Leu	
65					70					75					80	
Val	Gln	Glu	Gly	Ile	Val	Phe	Phe	Ser	Asp	Gln	Asp	Asp	Ile	Trp	Asp	
				85					90					95		
Cys	His	Lys	Ile	Glu	Thr	Met	Leu	Pro	Ile	Phe	Asp	Arg	Glu	Asn	Val	
			100					105					110			
Ser	Met	Val	Phe	Cys	Lys	Ser	Arg	Leu	Ile	Asp	Glu	Asn	Gly	Asn	Ile	
		115					120					125				
Ile	Ser	Ser	Pro	Asp	Thr	Ser	Asp	Arg	Ile	Asn	Thr	Tyr	Ser	Leu		
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<211> 3738

<212> DNA

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS7

<400> 43

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tgatgattgt tctaatacgc aaactgaaaa agttgtttcg catttcaaag attcaagaat 3300


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<210> 44

<211> 238

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS7E

<400> 44

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Val Lys Asn Asn Ile Phe Gly Thr Lys Asn Val Ala Glu Ala Ala Lys
20          25          30
Thr Ala Lys Val Ala Lys Phe Val Met Val Ser Thr Asp Lys Ala Val
35          40          45
Asn Pro Pro Asn Val Met Gly Ala Thr Lys Arg Val Ala Glu Met Ile
50          55          60
Val Thr Gly Leu Asn Glu Pro Gly Gln Thr Gln Phe Ala Ala Val Arg
65          70          75          80
Phe Gly Asn Val Leu Gly Ser Arg Gly Ser Val Val Pro Leu Phe Lys
85          90          95

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Glu	Gln	Ile	Arg	Lys	Gly	Gly	Pro	Val	Thr	Val	Thr	Asp	Phe	Arg	Met			
			100					105					110					
Thr	Arg	Tyr	Phe	Met	Thr	Ile	Pro	Glu	Ala	Ser	Arg	Leu	Val	Ile	Gln			
		115					120					125						
Ala	Gly	His	Leu	Ala	Lys	Gly	Gly	Glu	Ile	Phe	Val	Leu	Asp	Met	Gly			
	130					135					140							
Glu	Pro	Val	Gln	Ile	Leu	Glu	Leu	Ala	Arg	Lys	Val	Ile	Leu	Leu	Ser			
145					150					155					160			
Gly	His	Thr	Glu	Glu	Glu	Ile	Gly	Ile	Val	Glu	Ser	Gly	Ile	Arg	Pro			
				165					170					175				
Gly	Glu	Lys	Leu	Tyr	Glu	Glu	Leu	Leu	Ser	Thr	Glu	Glu	Arg	Val	Ser			
			180					185					190					
Glu	Gln	Ile	His	Glu	Lys	Ile	Phe	Val	Gly	Arg	Val	Thr	Asn	Lys	Gln			
		195					200					205						
Ser	Asp	Ile	Val	Asn	Ser	Phe	Ile	Asn	Gly	Leu	Leu	Gln	Lys	Asp	Arg			
	210					215					220							
Asn	Glu	Leu	Lys	Asp	Met	Leu	Ile	Glu	Phe	Ala	Lys	Gln	Glu					
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<210> 45

<211> 232

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS7F

<400> 45

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Ala	Thr	Ser	Lys	Tyr	Phe	Gln	Lys	Ile	Glu	Ser	Arg	Arg	Gly	Glu	Leu			
			20					25					30					
Phe	Ile	Lys	Phe	Phe	Met	Asp	Lys	Leu	Leu	Ala	Leu	Ile	Leu	Leu	Leu			
		35					40					45						

Leu Leu Ser Pro Val Ile Ile Ile Leu Ala Ile Trp Ile Lys Leu Asp
 50 55 60
 Ser Lys Gly Pro Ile Phe Tyr Arg Gln Glu Arg Val Thr Arg Tyr Gly
 65 70 75 80
 Arg Ile Phe Arg Ile Phe Lys Phe Arg Thr Met Ile Ser Asp Ala Asp
 85 90 95
 Lys Val Gly Ser Leu Val Thr Val Gly Gln Asp Asn Arg Ile Thr Lys
 100 105 110
 Val Gly His Ile Ile Arg Lys Tyr Arg Leu Asp Glu Val Pro Gln Leu
 115 120 125
 Phe Asn Val Leu Met Gly Asp Met Ser Phe Val Gly Val Arg Pro Glu
 130 135 140
 Val Gln Lys Tyr Val Asn Gln Tyr Thr Asp Glu Met Phe Ala Thr Leu
 145 150 155 160
 Leu Leu Pro Ala Gly Ile Thr Ser Pro Ala Ser Ile Ala Tyr Lys Asp
 165 170 175
 Glu Asp Ile Val Leu Glu Glu Tyr Cys Ser Gln Gly Tyr Ser Pro Asp
 180 185 190
 Glu Ala Tyr Val Gln Lys Val Leu Pro Glu Lys Met Lys Tyr Asn Leu
 195 200 205
 Glu Tyr Ile Arg Asn Phe Gly Ile Ile Ser Asp Phe Lys Val Met Ile
 210 215 220
 Asp Thr Val Ile Lys Val Ile Lys
 225 230

<210> 46

<211> 404

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS7G

<400> 46

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Ala	Glu	Ile	Asp	Glu	Val	Ile	Asp	Thr	Leu	Lys	Ser	Gly	Trp	Ile	Thr			
			20					25					30					
Thr	Gly	Pro	Lys	Thr	Lys	Glu	Leu	Glu	Arg	Arg	Leu	Ser	Val	Phe	Thr			
		35					40					45						
Gly	Thr	Asn	Lys	Thr	Val	Cys	Leu	Asn	Ser	Ala	Thr	Ala	Gly	Leu	Glu			
	50					55					60							
Leu	Val	Leu	Arg	Ile	Leu	Gly	Val	Gly	Pro	Gly	Asp	Glu	Val	Ile	Val			
65					70					75					80			
Pro	Ala	Met	Thr	Tyr	Thr	Ala	Ser	Cys	Ser	Val	Ile	Thr	His	Val	Gly			
				85					90					95				
Ala	Thr	Pro	Val	Met	Val	Asp	Ile	Gln	Lys	Asn	Ser	Phe	Glu	Met	Glu			
			100					105					110					
Tyr	Asp	Ala	Leu	Glu	Lys	Ala	Ile	Thr	Pro	Lys	Thr	Lys	Val	Ile	Ile			
		115					120					125						
Pro	Val	Asp	Leu	Ala	Gly	Ile	Pro	Cys	Asp	Tyr	Asp	Lys	Ile	Tyr	Thr			
	130					135					140							
Ile	Val	Glu	Asn	Lys	Arg	Ser	Leu	Tyr	Val	Ala	Ser	Asp	Asn	Lys	Trp			
145					150					155					160			
Gln	Lys	Leu	Phe	Gly	Arg	Val	Ile	Ile	Leu	Ser	Asp	Ser	Ala	His	Ser			
				165					170					175				
Leu	Gly	Ala	Ser	Tyr	Lys	Gly	Lys	Pro	Ala	Gly	Ser	Leu	Ala	Asp	Phe			
			180					185					190					
Thr	Ser	Phe	Ser	Phe	His	Ala	Val	Lys	Asn	Phe	Thr	Thr	Ala	Glu	Gly			
		195					200					205						
Gly	Ser	Val	Thr	Trp	Arg	Ser	His	Pro	Asp	Leu	Asp	Asp	Glu	Glu	Met			
	210					215					220							
Tyr	Lys	Glu	Phe	Gln	Ile	Tyr	Ser	Leu	His	Gly	Gln	Thr	Lys	Asp	Ala			
225				230						235					240			
Leu	Ala	Lys	Thr	Gln	Leu	Gly	Ser	Trp	Glu	Tyr	Asp	Ile	Val	Ile	Pro			
				245					250					255				
Gly	Tyr	Lys	Cys	Asn	Met	Thr	Asp	Ile	Met	Ala	Gly	Ile	Gly	Leu	Val			
			260					265					270					

Gln Leu Glu Arg Tyr Pro Ser Leu Leu Asn Arg Arg Arg Glu Ile Ile
 275 280 285
 Glu Lys Tyr Asn Ala Gly Phe Glu Gly Thr Ser Ile Lys Pro Leu Val
 290 295 300
 His Leu Thr Glu Asp Lys Gln Ser Ser Met His Leu Tyr Ile Thr His
 305 310 315 320
 Leu Gln Gly Tyr Thr Leu Glu Gln Arg Asn Glu Val Ile Gln Lys Met
 325 330 335
 Ala Glu Ala Gly Ile Ala Cys Asn Val His Tyr Lys Pro Leu Pro Leu
 340 345 350
 Leu Thr Ala Tyr Lys Asn Leu Gly Phe Glu Met Lys Asp Phe Pro Asn
 355 360 365
 Ala Tyr Gln Tyr Phe Glu Asn Glu Val Thr Leu Pro Leu His Thr Asn
 370 375 380
 Leu Ser Asp Glu Asp Val Glu Tyr Val Ile Glu Met Phe Leu Lys Ile
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 Val Ser Arg Asp

<210> 47

<211> 210

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> CPS7H

<400> 47

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 Met Pro Ser Trp Asn Thr Ala Lys Tyr Ile Ser Glu Ser Ile Gln Ser
 20 25 30
 Val Leu Asp Gln Thr His Gln Asn Trp Glu Leu Ile Ile Val Asp Asp
 35 40 45

Cys	Ser	Asn	Asp	Glu	Thr	Glu	Lys	Val	Val	Ser	His	Phe	Lys	Asp	Ser
50						55					60				
Arg	Ile	Lys	Phe	Phe	Lys	Asn	Ser	Asn	Asn	Leu	Gly	Ala	Ala	Leu	Thr
65					70					75					80
Arg	Asn	Lys	Ala	Leu	Arg	Lys	Ala	Arg	Gly	Arg	Trp	Ile	Ala	Phe	Leu
				85					90					95	
Asp	Ser	Asp	Asp	Leu	Trp	His	Pro	Ser	Lys	Leu	Glu	Lys	Gln	Leu	Glu
			100					105					110		
Phe	Met	Lys	Asn	Asn	Gly	Tyr	Ser	Phe	Thr	Tyr	His	Asn	Phe	Glu	Lys
		115					120					125			
Ile	Asp	Glu	Ser	Ser	Gln	Ser	Leu	Arg	Val	Leu	Val	Ser	Gly	Pro	Ala
	130					135						140			
Ile	Val	Thr	Arg	Lys	Met	Met	Tyr	Asn	Tyr	Gly	Tyr	Pro	Gly	Cys	Leu
145					150					155					160
Thr	Phe	Met	Tyr	Asp	Ala	Asp	Lys	Met	Gly	Leu	Ile	Gln	Ile	Lys	Asp
				165					170					175	
Ile	Lys	Lys	Asn	Asn	Asp	Tyr	Ala	Ile	Leu	Leu	Gln	Leu	Cys	Lys	Lys
			180					185					190		
Tyr	Asp	Cys	Tyr	Leu	Leu	Asn	Glu	Ser	Leu	Ala	Ser	Tyr	Arg	Ile	Arg
		195					200					205			
Lys	Lys														
	210														

<210> 48

<211> 101

<212> DNA

<213> Streptococcus suis

<220>

<221> misc_feature

<222> (1)..(101)

<223> N may be any nucleotide

<220>

<221> misc_feature
 <223> 100 base pair repeat between CPS2G and CPS2H
 <400> 48
 aagggcacct ctataaactc ccaaaattgc gaatttggag ttacgaaagc cttgttaaata 60
 caancatttt aaattttaga aaattagttt ttagagctcc c 101

 <210> 49
 <211> 101
 <212> DNA
 <213> Streptococcus suis
 <220>
 <221> misc_feature
 <222> (1)..(101)
 <223> N may be any nucleotide
 <220>
 <221> misc_feature
 <223> 100 base pair repeat within CPS2M
 <400> 49
 ggcgccacct ctataaattc ccaaaattgc gaatttcgag ttacgaaagc cttgttaaata 60
 caancatctt aaattttaga aaattagttt ttagaggctcc c 101

 <210> 50
 <211> 101
 <212> DNA
 <213> Streptococcus suis
 <220>
 <221> misc_feature

<223> 100 base pair repeat between CPS2O and CPS2P

<400> 50

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caaacattttt aaatttttaga aaatttagttt ttagagggtcc c 101

<210> 51

<211> 120

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> N-terminal part of CPS2J

<400> 51

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Leu	Arg	Glu	Cys	Leu	Asp	Ser	Ile	Ile	Ser	Gln	Ser	Tyr	Thr	Asn	Leu	
			20					25					30			
Glu	Ile	Leu	Leu	Ile	Asp	Asp	Gly	Ser	Ser	Asp	Ser	Ser	Thr	Asp	Ile	
		35					40					45				
Cys	Leu	Glu	Tyr	Ala	Glu	Gln	Asp	Gly	Arg	Ile	Lys	Leu	Phe	Arg	Leu	
	50					55					60					
Pro	Asn	Gly	Gly	Val	Ser	Asn	Ala	Arg	Asn	Tyr	Gly	Ile	Lys	Asn	Ser	
65					70					75					80	
Thr	Ala	Asn	Tyr	Ile	Met	Phe	Val	Asp	Ser	Asp	Asp	Ile	Val	Asp	Gly	
				85					90					95		
Asn	Ile	Val	Glu	Ser	Leu	Tyr	Thr	Cys	Leu	Lys	Glu	Asn	Asp	Ser	Asp	
		100						105					110			
Leu	Ser	Gly	Gly	Leu	Leu	Ala	Thr									
		115					120									

<210> 52

<211> 120

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> N-terminal part of CPS2K

<220>

<221> misc_feature

<222> (1)..(120)

<223> Xaa may be any amino acid

<400> 52

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Leu	Ser	Lys	Cys	Ile	Asn	Ser	Ile	Val	Asn	Gln	Thr	Tyr	Lys	His	Ile
			20					25					30		

Glu	Leu	Leu	Val	Asn	Asp	Gly	Ser	Ser	Thr	Asp	Asn	Ser	Glu	Glu	Ile
			35				40					45			

Cys	Leu	Ala	Tyr	Ala	Lys	Lys	Asp	Ser	Arg	Ile	Arg	Tyr	Phe	Lys	Lys
	50					55					60				

Glu	Asn	Gly	Gly	Leu	Ser	Asp	Ala	Arg	Asn	Tyr	Gly	Ile	Ser	Arg	Ala
65					70					75					80

Lys	Gly	Asp	Tyr	Leu	Ala	Phe	Ile	Asp	Ser	Asp	Asp	Phe	Ile	His	Ser
				85					90					95	

Glu	Phe	Ile	Gln	Arg	Leu	Xaa	His	Glu	Ala	Ile	Glu	Arg	Glu	Asn	Ala
			100					105					110		

Leu	Xaa	Xaa	Val	Ala	Val	Ala	Gly
		115					120

<210> 53

<211> 419

<212> PRT

<213> Streptococcus suis

<220>

<221> misc_feature

<223> ORF2Y

<400> 53

Met	Lys	Lys	Tyr	Gln	Val	Ile	Ile	Gln	Asp	Ile	Leu	Thr	Gly	Ile	Glu	
1				5					10					15		
Glu	His	Arg	Phe	Lys	Arg	Gly	Glu	Lys	Leu	Pro	Ser	Ile	Arg	Gln	Leu	
			20					25					30			
Arg	Glu	Gln	Tyr	His	Cys	Ser	Lys	Asp	Thr	Val	Gln	Lys	Ala	Met	Leu	
			35				40					45				
Glu	Leu	Lys	Tyr	Gln	Asn	Lys	Ile	Tyr	Ala	Val	Glu	Lys	Ser	Gly	Tyr	
	50					55					60					
Tyr	Ile	Leu	Glu	Asp	Arg	Asp	Phe	Gln	Asp	His	Thr	Cys	Arg	Ala	Gln	
65					70					75					80	
Ser	Tyr	Arg	Leu	Ser	Arg	Ile	Thr	Tyr	Glu	Asp	Phe	Arg	Ile	Cys	Leu	
				85					90					95		
Lys	Glu	Ser	Leu	Ile	Gly	Arg	Glu	Asn	Tyr	Leu	Phe	Asn	Tyr	Tyr	His	
			100					105					110			
Gln	Gln	Glu	Gly	Leu	Ala	Glu	Leu	Ile	Ser	Ser	Val	Gln	Ser	Leu	Leu	
			115				120					125				
Met	Asp	Tyr	His	Val	Tyr	Thr	Lys	Lys	Asp	Gln	Leu	Val	Ile	Thr	Ala	
	130					135					140					
Gly	Ser	Gln	Gln	Ala	Leu	Tyr	Ile	Leu	Thr	Gln	Met	Glu	Thr	Leu	Ala	
145					150					155					160	
Gly	Lys	Thr	Glu	Ile	Leu	Ile	Glu	Asn	Pro	Thr	Tyr	Ser	Arg	Met	Ile	
				165					170					175		
Glu	Leu	Ile	Arg	His	Gln	Gly	Ile	Pro	Tyr	Gln	Thr	Ile	Glu	Arg	Asn	
			180					185					190			
Leu	Asp	Gly	Ile	Asp	Leu	Glu	Glu	Leu	Glu	Ser	Ile	Phe	Gln	Thr	Gly	
		195					200						205			

Lys	Ile	Lys	Phe	Phe	Tyr	Thr	Ile	Pro	Arg	Leu	His	Asn	Pro	Leu	Gly
210						215					220				
Ser	Thr	Tyr	Asp	Ile	Ala	Thr	Lys	Thr	Ala	Ile	Val	Lys	Leu	Ala	Lys
225					230					235					240
Gln	Tyr	Asp	Val	Tyr	Ile	Ile	Glu	Asp	Asp	Tyr	Leu	Ala	Asp	Phe	Asp
				245					250					255	
Ser	Ser	His	Ser	Leu	Pro	Leu	His	Tyr	Leu	Asp	Thr	Asp	Asn	Arg	Val
			260					265					270		
Ile	Tyr	Ile	Lys	Ser	Phe	Thr	Pro	Thr	Leu	Phe	Pro	Ala	Leu	Arg	Ile
		275					280					285			
Gly	Ala	Ile	Ser	Leu	Pro	Asn	Gln	Leu	Arg	Asp	Ile	Phe	Ile	Lys	His
	290					295					300				
Lys	Ser	Leu	Ile	Asp	Tyr	Asp	Thr	Asn	Leu	Ile	Met	Gln	Lys	Ala	Leu
305					310					315					320
Ser	Leu	Tyr	Ile	Asp	Asn	Gly	Met	Phe	Ala	Arg	Asn	Thr	Gln	His	Leu
				325					330					335	
His	His	Ile	Tyr	His	Ala	Gln	Trp	Asn	Lys	Ile	Lys	Asp	Cys	Leu	Glu
			340					345					350		
Lys	Tyr	Ala	Leu	Asn	Ile	Pro	Tyr	Arg	Ile	Pro	Lys	Gly	Ser	Val	Thr
		355					360					365			
Phe	Gln	Leu	Ser	Lys	Gly	Ile	Leu	Ser	Pro	Ser	Ile	Gln	His	Met	Phe
	370					375					380				
Gly	Lys	Cys	Tyr	Tyr	Phe	Ser	Gly	Gln	Lys	Ala	Asp	Phe	Leu	Gln	Ile
385					390					395					400
Phe	Phe	Glu	Gln	Asp	Phe	Ala	Asp	Lys	Leu	Glu	Gln	Phe	Val	Arg	Tyr
				405					410					415	
Leu	Asn	Glu													

TABLE 1. Bacterial strains and plasmids

strain/plasmid	relevant characteristics	source/reference
Strain		
<i>E. coli</i>		
CC118	PhoA ⁻	(28)
XL2 blue	Stratagene	
<i>E. coli</i>		
XL2 blue	Stratagene	
<i>S. suis</i>		
10	virulent serotype 2 strain	(49)
3	serotype 2	(63)
17	serotype 2	(63)
735	reference strain serotype 2	(63)
T15	serotype 2	(63)
6555	reference strain serotype 1	(63)
6388	serotype 1	(63)
6290	serotype 1	(63)
5637	serotype 1	(63)
5673	serotype 1/2	(63)
5679	serotype 1/2	(63)
5928	serotype 1/2	(63)
5934	serotype 1/2	(63)
5209	reference strains serotype 1/2	(63)
5218	reference strain serotype 9	(63)

strain/plasmid	relevant characteristics	source/reference
5973	serotype 9	(63)
6437	serotype 9	(63)
6207	serotype 9	(63)
reference strains	serotypes 1-34	(9, 56, 14)
<i>S. suis</i>		
10	virulent serotype 2 strain	(51)
10cpsB	isogenic cpsB mutant of strain 10	this work
10cpsEF	isogenic cpsEF mutant of strain 10	this work
Plasmid		
pKUN19	replication functions pUC, Amp ^R	(23)
pGEM7Zf (+)	replication functions pUC, Amp ^R	Promega Corp.
pIC19R	replication functions pUC, Amp ^R	(29)
pIC20R	replication functions pUC, Amp ^R	(29)
pIC-spc	pIC19R containing spc ^R gene of pDL282	labcollection
pDL282	replication functions of pBR 322 and pVT736-1, Amp ^R , Spc ^R	(43)
pPHOS2	pIC-spc containing the truncated <i>phoA</i> gene of pPHO7 as a <i>Pst</i> I- <i>Bam</i> HI fragment	this work
pPHO7	contains truncated <i>phoA</i> gene	(15)
pPHOS7	pPHOS2 containing chromosomal <i>S. suis</i> DNA	this work
pCPS6	pKUN19 containing 6 kb <i>Hind</i> III fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS7	pKUN19 containing 3,5 kb <i>Eco</i> RI- <i>Hind</i> III fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS11	pCPS7 in which 0.4 kb <i>Pst</i> I- <i>Bam</i> HI fragment of <i>cpsB</i> gene is replaced by Spc ^R gene of pIC-spec	this work (Fig. 1)

strain/plasmid	relevant characteristics	source/reference
pCPS17	pKUN19 containing 3.1 kb <i>KpnI</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS18	pKUN19 containing 1.8 kb <i>SnaBI</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS20	pKUN19 containing 3.3 kb <i>XbaI-HindIII</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS23	pGEM7Zf (+) containing 1.5 kb <i>MluI</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS25	pIC20R containing 2.5 kb <i>KpnI-SalI</i> fragment of pCPS17	this work (Fig. 1)
pCPS26	pKUN19 containing 3.0 kb <i>HindIII</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS27	pCPS25 containing 2.3 kb <i>XbaI</i> (blunt) - <i>ClaI</i> fragment of pCPS20	this work (Fig. 1)
pCPS28	pCPS27 containing the 1.2 kb <i>PstI-XhoI</i> <i>Spc^R</i> gene of pIC-spc	this work (Fig. 1)
pCPS29	pKUN19 containing 2.2 kb <i>SacI-PstI</i> fragment of <i>cps</i> operon	this work (Fig. 1)
pCPS1-1	pKUN19 containing 5 kb <i>EcoRV</i> fragment of <i>cps</i> operon of type 1	this work (Fig. 1)
pCPS1-2	pKUN19 containing 2.2 kb <i>HindIII</i> fragment of <i>cps</i> operon of type 1	this work (Fig. 1)
pCPS9-1	pKUN19 containing 1 kb <i>HindIII-XbaI</i> fragment of <i>cps</i> operon of serotype 9	this work (Fig. 1)
pCPS9-2	pKUN19 containing 4.0 kb <i>XbaI-XbaI</i> fragment of <i>cps</i> operon of serotype 9	this work (Fig. 1)

Amp^R: ampicillin resistant
 Spc^R: spectinomycin resistant
 cps: capsular polysaccharide

Table 2. Properties of Orfs in the cps locus of *S.-suis*-*S. suis* serotype 2 and similarities to gene products of other bacteria

ORF	nucleotide position in sequence	number of amino acids	GC%	proposed function of gene product¹	similar gene product (% identity)
Orf2Z	1- 719	240	44	Unknown	<i>B. subtilis</i> YitS (26%)
Orf2Y	2079- 822	419	38	Transcription regulation	<i>B. subtilis</i> YcxD (39%)
Orf2X	2202- 2934	244	39	Unknown	<i>H. influenzae</i> YAAA (24%)
Cps2A	3041- 4484	481	39	Regulation	<i>S. pneumoniae</i> Cps19fA (58%)
Cps2B	4504- 5191	229	40	Chain length determination	<i>S. pneumoniae</i> type 3 Orf1 (58%)
Cps2C	5203- 5878	225	40	Chain length determination/ Export	<i>S. pneumoniae</i> Cps23fD (63%)
Cps2D	5919- 6648	243	38	Unknown	<i>S. pneumoniae</i> CpsB (62%)
Cps2E	6675- 8052	459	33	Glycosyltransferase	<i>S. pneumoniae</i> Cps14E (56%)
Cps2F	8089- 9256	389	32	Glycosyltransferase	<i>S. pneumoniae</i> Cps23fT
Cps2G	9262-10417	385	36	Glycosyltransferase	<i>S. thermophilus</i> EpsF (25%)
Cps2H	10808-12176	457	31	Glycosyltransferase	<i>S. mutans</i> RGPEC, ^N (29%)
Cps2I	12213-13443	410	29	CP polymerase	<i>S. pneumoniae</i> Cps23fI (48%)
Cps2J	13583-14579	332	29	Glycosyltransferase	<i>S. pneumoniae</i> Cps14J (31%)
Cps2K	14574-15576	334	37	Glycosyltransferase	<i>S. pneumoniae</i> Cps14J (40%)
“Cps2L”	15618-16635	103	37	Unknown	-
“Cps2M”	16811-17322	-	38	-	<i>S. agalactiae</i> CpsF ^N (77%) <i>E. coli</i> NeuA, ^N (47%)

ORF	nucleotide position in sequence	number of amino acids	GC%	proposed function of gene product ¹	similar gene product (% identity)
“Cps2N”	17559-18342	-	39	-	<i>S. agalactiae</i> CpsJ (43%)
Cps2O	18401-19802	476	40	Repeat unit transporter	<i>S. agalactiae</i> CpsK (41%)
Cps2P	20327-21341	338	39	Sialic acid synthesis	<i>S. agalactiae</i> NeuB (80%) <i>E. coli</i> NeuB (59%)
Cps2Q	21355-21865	170	42	Sialic acid synthesis	<i>S. agalactiae</i> NeuC ^N (61%) <i>E. coli</i> NeuC ^N (54%)
Cps2R	21933-22483	184	40	Sialic acid synthesis	<i>S. agalactiae</i> NeuC ^C (55%) <i>E. coli</i> NeuC ^C (40%)
Cps2S	22501-23125	208	42	Sialic acid synthesis	<i>E. coli</i> NeuD (32%)
Cps2T	23136-24366	395	40	CMP-NeuNAc synthetase	<i>S. agalactiae</i> CpsF (49%) <i>E. coli</i> NeuA (34%)
“Orf2U”	24566-25488	168	42	Transposase	<i>S. thermophilus</i> IS1194 (51%)
“Orf2V”	25691-26281	116	37	Transposase	<i>S. pneumoniae</i> orf1 (85%)

¹ Predicted by sequence similarity

^N Similarity refers to the amino-terminal part of the gene product

^C Similarity refers to the carboxy-terminal part of the gene product

ORFS between “ ” are truncated or non-functional as the result of frame-shift or point mutations

TABLE 3. Properties of ORFs in the cps gene of *S.-suis- S. suis* serotypes 1 and 9 and similarities to gene products of other bacteria

ORF	nucleotide position in sequence	G + C%	number of amino acids	predicted mol. mass (kDa)	predicted pI	proposed function of gene product ¹	similar gene product (%) identity)	reference/ accession nr.
Cps1E ²	1-1363	34%	454	52.2	8.0	Glucosyltransferase	<i>Streptococcus suis</i> Cps2E (86%)	(26)
Cps1F	1374-1821	33%	149	17.3	8.2	Unknown	<i>Streptococcus pneumoniae</i> Cps14E (48%)	(12)
Cps1G	1823-2315	25%	164	19.5	7.5	Glycosyltransferase	<i>Streptococcus pneumoniae</i> Cps14F (83%)	(14)
Cps1H	3035-4202	24%	389	45.5	8.4	CP polymerase	<i>Streptococcus pneumoniae</i> Cps14G (50%)	(14)
Cps1I	4917-					Glycosyltransferase	<i>Streptococcus pneumoniae</i> Cps14H (30%)	(14)
							<i>Streptococcus pneumoniae</i> Cps14J (38%)	(13)
							<i>Lactococcus lactis</i> EpsG (31%)	(29)
							<i>Streptococcus thermophilus</i> EpsI (33%)	(28)
Cps1J						Glycosyltransferase	<i>Streptococcus pneumoniae</i> Cps14J (%)	(13)

ORF	nucleotide position in sequence	G + C%	number of amino acids	predicted mol. mass (kDa)	predicted pI	proposed function of gene product ¹	similar gene product (% identity)	reference/ accession nr.
Cps1K ³		37%	278	32.5	7.8	Glycosyltransferase	<i>Streptococcus pneumoniae</i> Cps14J (44%)	(13)
Cps9D ²	1-646	37%	215	24.9	8.1	Unknown	<i>Streptococcus suis</i> Cps2D (89%)	(26)
Cps9E	680-					Glycosyltransferase	<i>Staphylococcus aureus</i> Cap1D (27%)	(18)
Cps9F		36%	200	22.3	8.2	Glycosyltransferase	<i>Staphylococcus aureus</i> Cap5M (52%)	(17)
Cps9G		35%	269	31.5	8.0	Unknown	<i>Actinobacillus actinomycetemcomitans</i> (43%)	(AB002668_4)
							<i>Haemophilus influenzae</i> Lsg (43%)	(O05081)
Cps9H ³		30%	143	16.5	7.2	Unknown	<i>Yersinia enterocolitica</i> RfbB (28%)	(33)

¹ Predicted by sequence similarity

² N-terminal part of protein is lacking

³ C-terminal part of protein is lacking

Table 4. Hybridization of serotype 2 cps genes and neighboring sequences with chromosomal DNA of serotypes

serotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	1/2		
DNA probes																																					
<i>orf2Z</i>	+	+	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	-	+		
<i>orf2Y</i>	+	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+	+	+	±	+	±	-	+	+	+	+	+	-	+	+	+	+	-	-	+		
<i>orf2X</i>	+	+	+	+	+	+	+	+	+	+	+	±	±	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+		
<i>cps2A</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+		
<i>cps2B</i>	+	+	+	+	+	+	+	+	+	+	-	-	±	+	-	-	±	±	±	±	-	±	-	+	+	+	-	-	+	+	±	+	-	±	+		
<i>cps2C</i>	+	+	+	+	+	+	+	+	+	+	+	-	±	+	-	-	±	-	-	-	-	-	+	+	+	+	-	+	±	+	+	-	-	+			
<i>cps2D</i>	+	+	+	+	+	+	+	+	+	+	+	±	±	+	-	-	±	+	+	±	-	-	+	+	+	-	+	+	+	±	+	-	-	+			
<i>cps2E</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+			
<i>cps2F</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>cps2G</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	±	+			
<i>cps2H</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>cps2I</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>cps2J</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>cps2K</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
“ <i>cps2L</i> ”	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
“ <i>cps2M</i> ”	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+			
<i>cps2N</i> ”	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+			
<i>cps2O</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+			
<i>cps2P</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+			
<i>cps2Q</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+			
<i>cps2R</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+			
<i>cps2S</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+			

serotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	1/2
<i>cps2T</i>	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
“ <i>orf2U</i> ”	+	+	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+
“ <i>orf2V</i> ”	+	+	±	±	±	-	±	-	-	-	-	-	-	+	+	-	+	±	-	-	-	±	+	-	-	+	-	-	-	-	+	+	-	±	+
<i>100-bp repeat</i>	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	-	+	-	+	+
<i>16SrRNA</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 5. Hybridization of serotypes 1 and 9 *cps* genes with chromosomal DNA of other *S. suis* serotypes

Serotype	DNA probes									
	<i>cps1E</i>	<i>cps1F</i>	<i>cps1G</i>	<i>cps1H</i>	<i>cps1I</i>	<i>cps9E</i>	<i>cps9F</i>	<i>cps9G</i>	<i>cps9H</i>	16rRNA
1	+	+	+	+	+	-	-	-	-	-
2	+	-	-	-	-	-	-	-	-	+
3	-	-	-	+	-	+	-	-	-	+
4	-	-	-	+	-	+	-	-	-	+
5	-	-	-	+	-	+	-	-	-	+
6	-	-	-	-	-	-	-	-	-	+
7	-	-	-	+	-	+	-	-	-	+
8	-	-	-	-	-	-	-	-	-	+
9	-	-	-	+	-	+	+	+	+	+
10	-	-	-	+	-	+	+	-	-	+
11	-	-	-	+	-	+	±	-	-	+
12	-	-	-	±	-	+	±	-	-	+
13	-	-	-	+	-	+	-	-	-	+
14	+	+	+	+	+	-	-	-	-	+
15	-	-	-	-	-	-	-	-	-	+

Serotype	<i>cps1E</i>	<i>cps1F</i>	<i>cps1G</i>	<i>cps1H</i>	<i>cps1I</i>	<i>cps9E</i>	<i>cps9F</i>	<i>cps9G</i>	<i>cps9H</i>	16rRNA
16	-	-	-	-	-	-	-	-	-	+
17	-	-	-	+	-	+	-	-	-	+
18	-	-	-	+	-	+	-	-	-	+
19	-	-	-	+	-	+	-	-	-	+
20	-	-	-	-	-	-	-	-	-	+
21	-	-	-	+	-	+	±	-	-	+
22	-	-	-	-	-	-	-	-	-	+
23	-	-	-	+	-	+	-	-	-	+
24	-	-	-	+	-	+	+	-	-	+
25	-	-	-	-	-	-	-	-	-	+
26	-	-	-	-	-	-	±	-	-	+
27	+	-	-	-	-	-	-	-	-	+
28	-	-	-	+	-	+	±	-	-	+
29	-	-	-	+	-	+	-	-	-	+
30	-	-	-	+	-	+	±	-	-	+
31	-	-	-	+	-	+	-	-	-	+
32	-	-	-	-	-	-	-	-	-	+
33	-	-	-	-	-	-	±	-	-	+
34	-	-	-	-	-	-	-	-	-	+
1/2	+	-	-	-	-	-	-	-	-	+

TABLE 6. Virulence of ~~wild-type~~ wild-type and capsular mutant *S. suis* strains in germfree pigs

<i>S. suis</i> strains ¹	pigs/ group (n)	mortality ² (%)	morbidity ³ (%)	clinical index of the group	spec symptoms ⁵	non-spec. symptoms ⁶	fever index ⁷	leucocyte index ⁸	isolation of <i>S. suis</i> in pigs (n) per group in	CNS	serosae	joints
10	4	100	100	11	88		43	44	2	3		4
10cpsB	4	0	0	0	10		1	3	1	3		2
10cpsEF	4	0	0	0	0		1	0	1	3		2

¹ strain10 in the ~~wild-type~~ wild-type strain, strains 10cpsB and 10cpsEF are isogenic capsular mutant strains

² piglets which died spontaneously or had to be killed for animal welfare reasons

³ only considering pigs with specific symptoms

⁴ clinical index: % of observations which matched the described criteria

⁵ specific symptoms: ataxia, ~~lethargy~~ lameness on at least one joint, stiffness

⁶ non-specific symptoms: inappetence, depression

⁷ % of observations in the experimental group with a body temperature > 40°C

⁸ % of blood samples in the group in which number of granulocytes > 10¹⁰/l

Table 7. Bacterial strains and plasmids

strain/plasmid	relevant characteristics
Strain	
<i>E. coli</i>	
XL2 blue	
<i>S. suis</i>	
reference strains	
5667	serotypes 1-34
7037	serotype 7, tonsil (1993)
7044	serotype 7, organs (1994)
7068	serotype 7, brains (1994)
7646	serotype 7 (1994)
7744	serotype 7, lungs (1996)
7759	serotype 7, joints (1996)
8169	serotype 7 (1997)
15913	serotype 7, meninges (1998)
Plasmid	
pKUN19	replication functions pUC, Amp ^R
pGEM7Zf (+)	replication functions pUC, Amp ^R
pCPS9-1	pKUN19 containing 1 kb <i>Hind</i> III- <i>Xba</i> I fragment of <i>cps</i> operon of serotype 9
pCPS9-2	pKUN19 containing 4.09 kb <i>Xba</i> I- <i>Xba</i> I fragment of <i>cps</i> operon of serotype 9
pCPS7-1	pKUN19 containing 1.6-kb <i>Pst</i> I fragment of <i>cps</i> operon of type 7
pCPS7-2	pGEM7 containing 2.7-kb <i>Sca</i> I- <i>Cl</i> aI fragment of <i>cps</i> operon of type 7

Amp^R: ampicillin resistant

cps: capsular polysaccharide

Table 8. Properties of Orfs in the *cps* genes of *S. suis* serotype 7 and similarities to gene products of other bacteria

Orf	nucleotide position in sequence	proposed function of gene product	similar gene product (% identity)
Cps7E	1-719	Glycosyltransferase	<i>Streptococcus suis</i> Cps9E (99%)
Cps7F	1164-1863	Glycosyltransferase	<i>Bordetella pertussis</i> BpIG ¹ (43%) <i>Streptococcus suis</i> Cps2E ¹ (33%)
Cps7G	1872-3086	Biosynthesis amino sugar	<i>Bordetella pertussis</i> BpIF (48%)
Cps7H	3104-3737	Glycosyltransferase	<i>Escherichia coli</i> WbdN (35%) <i>Streptococcus suis</i> Cps2K ² (31%)

¹ similarity refers to the C-terminal part of the gene product

² similarity refers to the N-terminal part of the gene product

Table 9. Hybridization of serotype 7 *cps* probes with chromosomal DNA of *S. suis* serotypes

serotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	1/2			
DNA probes																																						
<i>cps7E</i>	-	-	+	+	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	-	-	-	+	+	-	-	-	-	-			
<i>cps7F</i>	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-			
<i>cps7G</i>	-	-	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-			
<i>cps7H</i>	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
<i>I6SrRNA</i>	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			

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~~Abstract of the Disclosure~~ ABSTRACT OF THE DISCLOSURE

The invention relates to *Streptococcus suis* infection in pigs, vaccines directed against those infections and tests for diagnosing *Streptococcus suis* infections. The invention provides an isolated or recombinant nucleic acid encoding a capsular gene cluster of *Streptococcus suis* or a gene or gene fragment ~~derived~~ derived thereof. The invention further provides a nucleic acid probe or primer allowing species or ~~serotype specific~~ serotype-specific detection of *Streptococcus suis*. The invention also provides a *Streptococcus suis* antigen and vaccine derived thereof.

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